FACTORS INFLUENCING SECONDARY BILE ACID FORMATION IN MAN

with reference to colonic carcinogenesis

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WITH REFERENCE TO COLONIC CARCINOGENESIS

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Introduction and outline of the studies

Colonic carcinogenesis in man seems to be related to dietary habits. A high dietary fat and low fibre intake is associated with a high prevalence of colonic cancer. Epidemiological investigations cannot clarify whether this association is causal and if so, by which mechanisms these dietary factors exert their action. An aspect of diet, which is regarded important is the influence on secondary bile acid formation in the large bowel. Secondary bile acids have been incriminated in colonic carcinogenesis, since it has been shown that these compounds have tumour promoting capacities in animal models, are co-mutagenic and enhance colonic mucosal proliferation. The concentration of secondary bile acids in the large bowel seems to be associated with the risk of developing colonic cancer.
The main purpose of the studies presented in this thesis is to extend the knowledge about factors that modulate secondary bile acid formation.
In chapter I a review is given on the possible role of bile acids in colonic carcinogenesis. Chapter II describes the methodology of faecal bile acid analysis. In chapter III it is shown that faecal bile acid concentration is age-dependent and related to changes in fibre consumption with advancing age. Chapter IV provides evidence that secondary bile acid formation can be reduced by acidification of colonic contents. The effect of a natural high fibre diet on stool characteristics and biliary and faecal bile acid profiles are presented in chapter V. In chapter VI the relationship between the molar percentages of deoxycholic acid in bile and serum is presented.

CHAPTFR I

Bile acids and colonic carcinogenesis

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CHAPTER I

Bile acids and colonic carcinogenesis

Abstract

Dietary factors are considered important environmental risk determinants for colorectal cancer development. From epidemiological observations a high fat intake is associated positively and a high fibre intake negatively with colorectal cancer. One mechanism by which these effects are possibly exerted is through bile acid metabolism. Secondary bile acids are formed after enzymatic deconjugation and dehydroxylation of the primary bile acids in the large bowel by anaerobic bacteria. It has been shown that these secondary bile acids can have tumour promoting capacities. They are co-mutagenic and have shown co-carcinogenic properties in animal studies. Furthermore, secondary bile acids can enhance colonic epithelial proliferation. In populations and patients at high risk for developing colorectal cancer higher faecal concentrations and colonic absorption of secondary bile acids can be found. The formation of secondary bile acids increases with advancing age. Dietary fat increases and dietary fibre reduces the concentration of bile acids in the large bowel. Inhibition of secondary bile acid formation can be achieved by acidification of colonic contents.
In colorectal carcinogenesis, environmental factors such as dietary habits influence genetic susceptibility. Secondary bile acids could play a promoting role in this process.

Introduction

Bile acids are the major end products of cholesterol metabolism. The primary bile acids cholic acid (CA) and chenodeoxycholic acid (CDCA) are secreted into bile as glycine or taurine conjugates. They serve as cholesterol solubilizing agents and play an important role in the digestion and absorption of lipids in the small intestine. More than 95% of the bile acids, passing the terminal ileum are reabsorbed and returned to the liver. Efficient conservation of the bile acids in the enterohepatic circulation is thus maintained. The proportion of bile acids not absorbed in the small intestine is 2-3% per cycle and amounts to an average loss of 20% of the primary bile acid pool with 6-12 enterohepatic circulations per day (giving a primary bile acid load to the large bowel of approximately 1.5-2.0 mmol/day). The bile acids that escape absorption in the ileum, are metabolized by the anaerobic

colonic flora. Thus, after deconjugation the primary bile acids CA and CDCA are dehydroxylated and converted into the secondary bile acids deoxycholic acid (DCA) and lithocholic acid (LCA) respectively. Further bacterial degradation and alterations in the liver produces tertiary bile acids (see figure 1). DCA is partly absorbed in the (proximal) colon and enters the enterohepatic circulation (about 0.2-0.5 mmol/day). LCA is almost entirely insoluble and very little is absorbed. In the circulating bile acid pool, CA and CDCA each comprise about 30-40%, DCA 20-30%, and LCA accounts for only 1-3% of the total. The bile acids not absorbed in the ileum and colon are excreted in the faeces. More than 90% of the bile acids excreted (about 0.5-1 mmol/day) are the unconjugated secondary bile acids DCA and LCA (1, 2). In figure 2 the enterohepatic circulation of bile acids is shown.

The secondary bile acids DCA and LCA are suspected to play a promoting role in colonic carcinogenesis, and lines of evidence and possible mechanisms will be discussed in this review.

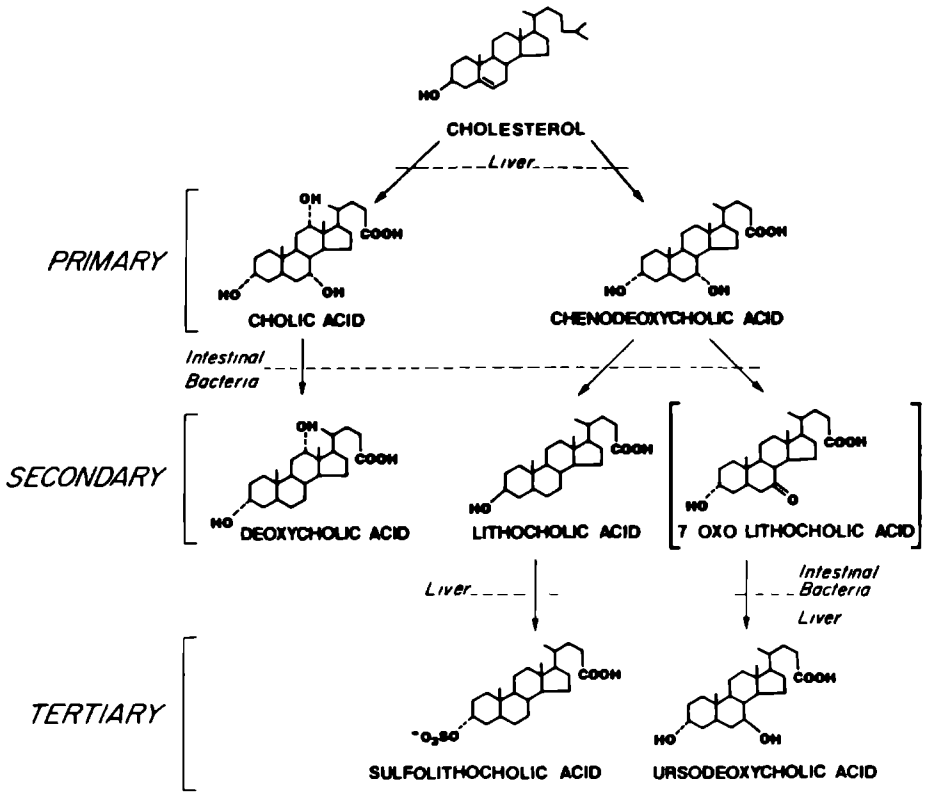

Figure 1 Synthesis and transformation of the major bile acids from cholesterol.

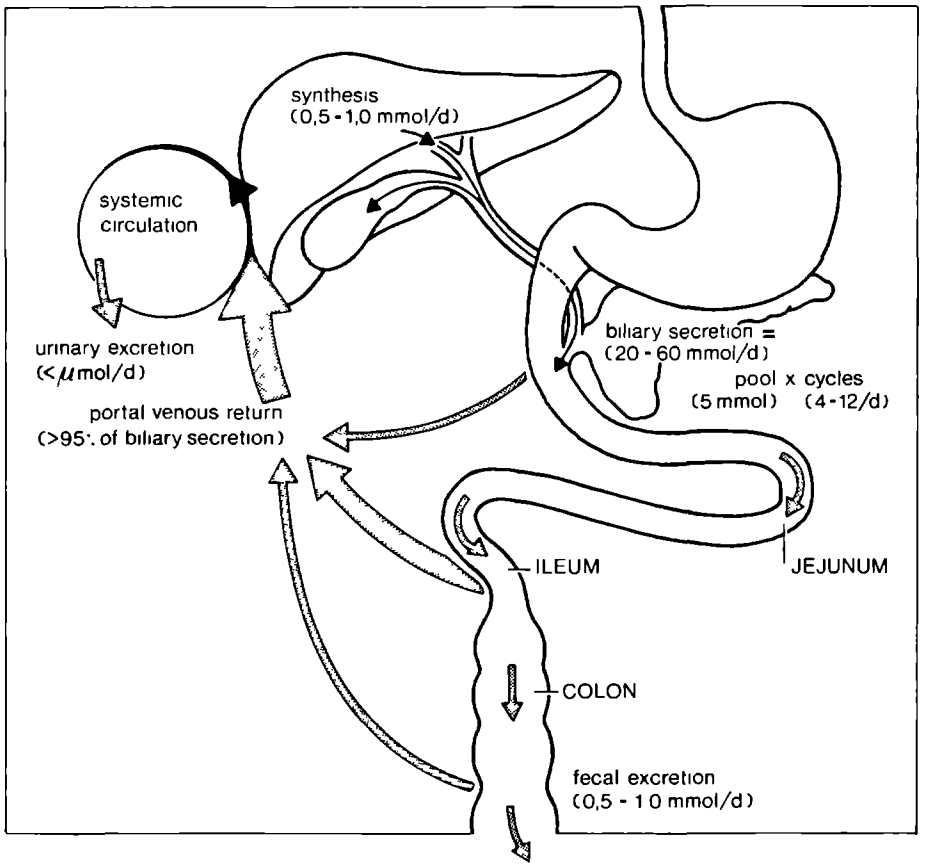

Figure 2 The enterohepatic circulation of bile acids in man

Epidemiological observations

The incidence of colonic cancer is high in Western countries, but low in Africa. Annual incidence rates vary from 1.3 per 100.000 inhabitants in Nigeria to 30 per 100.000 in parts of the USA and Scotland (3-5). The annual mortality (per 100.000 inhabitants) in the Netherlands in 1986 for colorectal cancer was 25.8 for males and 27.7 for females (6). Dietary factors are considered to be among the most important risk determinants for large bowel carcinogenesis (7). In this respect a diet high in animal fat is held responsible for an increased risk and consumption of a fibre-rich diet is considered to be protective (7,8-14). Migrant studies revealed that migrants from low-incidence to high-incidence areas acquired a higher risk for large bowel cancer, with an increase in risk apparent already in the first generation (10, 15-17). Furthermore, within a given high risk population

groups with different life styles have different incidences of colonic cancer. Mormons and Seventh Day Adventists in the USA have a lower incidence (18-19). They consume a diet low in fat and high in fibre. A likely explanation of the differences observed is the divergent dietary composition. Besides the influence of fat and fibre, other dietary constituents such as trace elements (selenium) and vitamins have been suggested to play a role and have a protective effect (7).

Fat

The hypothesis postulates that a high fat diet enhances the formation and degradation of bile acids and neutral sterols and that these compounds exert a promoting or cocarcinogenic effect on the large bowel mucosa (20). Indeed, it has been found that dietary fat increases the output and faecal concentration of bile acids (21-24) and epidemiological evidence has shown that populations on a high fat and animal protein diet excrete twice the amount of secondary bile acids (7, 25-28). The concentration of these bile acids was even more increased. However, studies on dietary consumption patterns with time in the United States, England, Australia and New Zealand have failed to demonstrate a correlation between a high fat, high protein intake and colorectal cancer incidence (29, 30). Case control studies concerning the relation of diet and large bowel cancer have given conflicting results (19, 31-33), showing a positive association between fat and protein consumption and large bowel cancer in some (19, 31), but no association in other studies (32, 33).

Fibre

A high dietary fibre intake possibly protects against colorectal carcinogenesis (7, 20). Epidemiological studies have shown a negative correlation between dietary fibre consumption and large bowel cancer incidence (26-28, 30). Also, case-control studies have demonstrated a significantly lower fibre consumption in cancer patients (35-37). The effect is thought to act via several mechanisms: dilution of the colonic contents by water-retaining capacities of certain parts of the fibres; binding of promoting substances like bile acids; accelerating intestinal transit, leaving less contact time between (co)carcinogens and the bowel mucosa and fermentation of hemicellulose and other components of fibre with the production of short chain fatty acids which reduce the colonic pH. This increase in acidification of the proximal large bowel contents can influence bacterial metabolism and suppress the formation of secondary bile acids or reduce their solubility (38-40). Different parts of fibres probably have divergent effects, but in the whole a diet rich in fibre reduces the faecal concentration of bile acids. The total output of bile acids can increase but often does not change (38-40). The Finnish population has a low incidence of colonic cancer despite a rather high fat intake. It has been

shown that the lower incidence correlates well with the high fibre intake of this population (26, 27, 34). Faecal bile acid concentrations in Finland (especially in rural areas) were much lower than those in other parts of Scandinavia or the United States. A protective role, not for the total dietary fibre intake but for the pentose fraction, was assumed by Bingham et al. (41).

Experimental evidence

The hypothesis that the relation between diet and colorectal cancer incidence is established via bile acid metabolism has led to many experimental studies in animal models, on the cellular level and in man.

Animal experiments

Secondary bile acids can act as tumour promoters in animal experiments. Because spontaneous colorectal cancer rarely occurs in animals, initiating carcinogens such as azoxymethane and 1,2-dimethyldihydrazine have to be used. Studies have been performed both by dietary manipulation (with fat and fibre) and by direct application of bile acids to the colonic mucosa (42-50). Most studies have been carried out in rodent models (rat, mouse). It has been shown that DCA and LCA have tumourpromoting capacities and that this effect is also present in germfree animals; further degradation is therefore not a prerequisite (45, 46). The primary bile acids have a much less pronounced effect on carcinogenesis (46).

Feeding rats high fat diets resulted in a higher tumour yield and an increased faecal bile acid concentration (42, 43). Addition of fibre can reduce the tumour yield (48, 49), although, results were contradictory in this respect (50).

Mutagenity studies

Invitro testing by the Salmonella mutagenit assay (Ames test) demonstrated that the secondary bile acids exert comutagenic effects, but are not mutagens themselves (51-53). LCA can break DNA strands in a cultured L1210 cell line as well (54). In the hamster embryo cell transformation assay LCA, but not its sulphate ester was mutagenic (55). It has recently been demonstrated that both the concentration of secondary bile acids and a high faecal mutagen excretion in man could be reduced by suppletion of extra fibre to the diet (56).

Proliferative activity

It has been postulated that an increased colonic epithelial proliferative activity is related to an increased risk for the development of cancer (57). In the normal mucosa proliferative activity resides in the lower and middle regions of the colo-

nic crypts. During enhanced proliferative activity, epithelial cells show an increased ability to proliferate and accumulate in the upper parts of the mucosa. This proliferation can be measured by the [^{3}H]-thymidine labelling index (57-59) and recently by a new assay measuring the ornithine decarboxylase activity in the mucosa (60). This last enzyme is rate-limiting in the synthesis of polyamines, which play an important role in the proliferation of intestinal cells (61, 62). Secondary bile acids have been shown to enhance proliferative activity both in animal and human epithelial cells (63-68). It has been suggested that the concentration of the bile acids in the faecal water fraction (e.g. the soluble fraction) is the major determinant of this effect (69-70). The effect is possibly inhibited by calcium (71) and it has been suggested that adding extra calcium to the diet could be beneficial in the prevention of large bowel cancer (72). Data, however, are still incomplete and more studies are needed before firm conclusions can be drawn in this respect (69).

Human studies

Since epidemiological population-based studies incriminated bile acids in the aetiology of colorectal cancer, several investigations in man have been carried out. Most studies were case-control experiments in which the faecal bile acid concentration and excretion was the main parameter. Subjects were patients with large bowel cancer or persons at a high risk for developing this tumour (e.g. patients with colonic adenomas, longstanding ulcerative colitis or with a familial occurrence of the tumour). Controls were healthy subjects as well as patients with non malignant bowel diseases or non gastrointestinal diseases. The results of all these studies were conflicting: in some a higher secondary faecal bile acid concentration was shown in patients with colorectal cancer or subjects at higher risk (73, 74). However, most studies did not reveal any difference (75-80) or even demonstrated a higher faecal bile acid concentration in patients without colorectal cancer (81). This discrepancy is probably due partly to the fact that age and dietary intake of fat and fibre were not controlled in most of these investigations. Faecal bile acid concentration proves to be age-dependent and related to the dietary intake of fibre (82).

In some studies biliary bile acids were measured or bile acid kinetic studies were carried out. The absorption of DCA in the colon proved to be age-dependent as well (83), to be higher in adenoma patients than in age-matched controls, and to coincide with a more anaerobic environment (84, 85). Recently, a larger fraction of CDCA in bile was shown in adenoma and carcinoma patients than in controls (86). Owen et al (87) have suggested that not only the secondary bile acid fraction in faeces is a determinant of the risk of colorectal cancer. They found a signifi-

cantly higher LCA/DCA ratio in stools of high risk subjects, suggesting an increased synthesis rate of CDCA over CA in the liver and thus in agreement with the increase of CDCA in bile in adenoma and carcinoma patients. However, interpretations must be made with caution, because in these last studies no kinetic experiments were carried out.

Cholecystectomy and colorectal cancer

Patients with a cholecystectomy have a higher proportion of DCA in their enterohepatic circulation, probably due to a more continuous and frequent enterohepatic circulation and therefore exposure of a larger quantity of bile acids to the colonic flora (1). It must be noted in this respect that cholelithiasis itself can be associated with an increase in DCA in bile (88-91). In animal experiments cholecystectomy led to an increase in colonic tumours in one but not in another study (92,93). In man, the relation between cholecystectomy and the development of colorectal cancer has been studied quite intensively in retrospective case-control studies. Results varied from an increase in risk especially in the proximal colon in women, to no difference in the groups studied (94). Two "prospective" studies evaluating patients with a cholecystectomy were also contradictory: in a Mayo Clinic study an increased risk was found (95), whereas a Swedish study revealed no increased cancer risk (96). Reviewing the data so far obtained, no firm conclusions can be drawn. Possibly a common dietary factor predisposing to gallstone disease and thus to cholecystectomy is also implicated in colonic carcinogenesis. It cannot be ruled out that an increased risk for developing rightsided colonic cancer, especially in females, does exist after cholecystectomy.

Modulation of secondary bile acid metabolism

Theoretically, inhibition of the formation of secondary bile acids in the large bowel should lead to a lower colonic cancer risk. In animal experiments manipulation of diet with dietary factors such as fibre can indeed decrease the tumour yield and secondary bile acid formation. In man, faecal mutagenity and faecal bile acid concentration were inhibited by adding extra fibre to the diet (56). Lactulose can inhibit DCA formation and absorption by reducing colonic pH and possibly by an effect on intestinal transit time (97, 98).
Nevertheless, it is not clear at this moment whether the inhibition of secondary bile acid formation really reduces the risk of the development of colonic cancer in man and which role dietary habits play in this process. Prospective dietary intervention studies now in progress (69) may give more insight into this issue.

Role of bile acids in colonic carcinogenesis

Both epidemiological observations and experimental studies in animals have shown that secondary bile acids are possibly involved in colorectal carcinogenesis.

The diverging results of case-control studies are partly related to methodological problems and inadequate matching for dietary habits and age between cases and controls. It has recently been shown that bile acids can influence the proliferative activity of the colonic epithelial cells, and this effect can be modulated by dietary means (69). Surely, if involved, bile acids are only one factor in the causation of this tumour and many other intraluminal and systemic factors contribute. The intraluminal pH in the large bowel could play a key role in this respect. A high colonic pH favours the degradation of many compounds (e.g. bile acids) while these reactions are inhibited at lower pH values. In colorectal carcinogenesis, environmental factors such as dietary habits probably influence genetically determined susceptibility. Secondary bile acids could play a promoting role in this process (see figure 3).

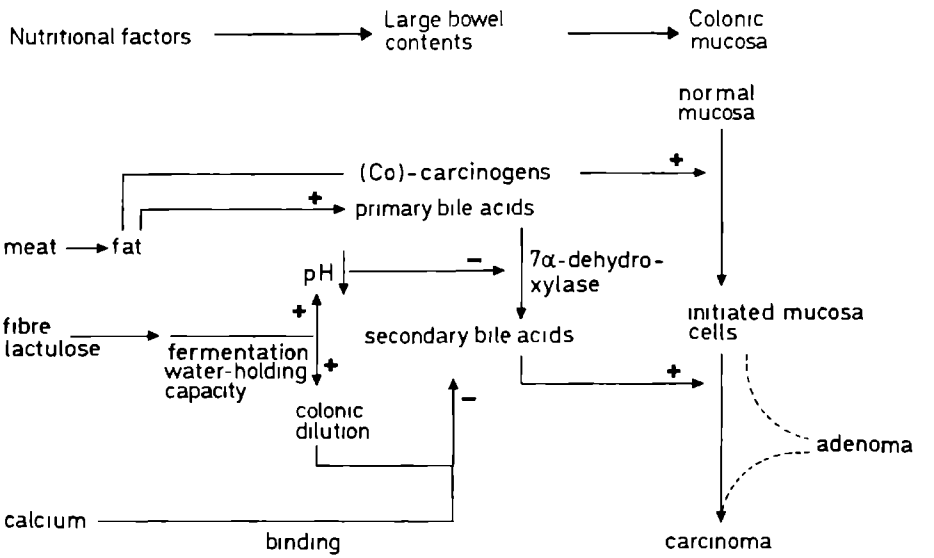

Figure 3 Hypothesis colonic carcinogenesis in man, with special reference to the role of secondary bile acids

References

1 Carey MC The enterohepatic circulation In Arias I, Popper H, Schachter D and Shafritz DA eds The liver biology and pathobiology Raven Press, New York 1982 429 65
2 MacDonald IA, Bokkenheuser VD, Winter J, McLernon AM, Mosbach EH Degradation of steroids in the human gut J Lipid Res 1983, 24 675 700
3 Burkitt DP Epidemiology of cancer of the colon and rectum Cancer 1971, 28 3-13
4 Doll R The geographical distribution of cancer Br J Cancer 1969, 23 1 8
5 Lambert R Epidemiology of colorectal carcinogenesis In Malt RA, Williamson RCL, eds Colonic carcinogenesis MTP Press Limited, Lancaster Boston The Hague 1982 1-10
6 CBS figures, The Netherlands, 1986
7 Zaridze DG Environmental etiology of large bowel cancer J Natl Cancer Inst 1983, 70 389-400
8 Armstrong GB, Doll R Environmental factors and cancer incidence and mortality in different countries with special reference to dietary practices Int J Cancer 1975, 15 613-31
9 Burkitt DP Colonic rectal cancer fiber and other dietary factors Am J Clin Nutr 1978, 31 S58 64
10 Haenszel W, Berg JW, Segi M, Kurihara M, Locke FB Large bowel cancer in Hawaiian Japanese J Natl Cancer Inst 1973, 51 1765 79
11 McKeownEyssen GE, BrightSee E Dietary factors in colon cancer international relationships Nutr Cancer 1984, 6 160 70
12 Wynder EL Dietary habits and cancer epidemiology Cancer 1979, 43 1955-61
13 Kritchevsky D Dietary fiber and cancer Nutr Cancer 1985, 6 213 19
14 Weisburger JH Role of fat, fiber, nitrate and food additives in carcinogenesis a critical evaluation and recommendations Nutr Cancer 1986, 8 47-62
15 Haenszel W, Kurihara M Studies of Japanese migrants I Mortality from cancer and other diseases among Japanese in the United States J Natl Cancer Inst 1968, 40 43-68
16 Smith RI Recorded and expected mortality among Japanese of the United States and Hawaii, with special reference to cancer J Natl Cancer Inst 1956, 17 459 73
17 Staszewski J, McCall MG, Stenhouse NS Cancer mortality in 1962 1966 among Polish migrants to Australia Br J Cancer 1971, 25 599 610
18 Lyon JL, Klauber MR, Gardner JW, Smart CR Cancer incidence in Mormons and non-Mormons Utah 1966-1970 N Engl J Med 1976, 294 129-33
19 Phillips RL Role of lifestyle and dietary habits in risk of cancer among Seventh-Day Adventists Cancer Res 1975, 35 3513-22
20 Breuer N, Goebell H Bile acids and colonic cancer Klin Wochenschr 1985, 63 97-105
21 Low-Beer TS Diet and bile acid metabolism Clin Gastroenterol 1977, 6 165 78
22 Cummings JH, Wiggins HS, Jenkins DJA, Houston H, Jivraj T, Drasar BS, Hill MJ Influence of diets high and low in animal fat on bowel habit, gastrointestinal transit time, fecal microflora, bile acid and fat excretion J Clin Invest 1978, 61 953 63
23 Hill MJ The effect of some factors on the faecal concentration of acid steroids, neutral steroids and urobilins J Pathol 1971, 104 239-45
24 Reddy BS, Weisburger HJ, Wynder FL Effects of high risk and low risk diets for colon carcinogenesis on fecal sterol microflora and steroids J Nutr 1975, 105 878-84
25 Reddy BS, Fkelund G, Bohe M, Engle A, Domellof I Metabolic epidemiology of colon cancer dietary pattern and fecal sterol concentrations of three populations Nutr Cancer 1983, 5 34-40
26 Reddy BS, Hedges A, Laakso K, Wynder EL Fecal constituents of a highrisk North American and lowrisk Finnish population for the development of large bowel cancer Cancer 1978, 42 2832 8
27 Jensen OM, MacLennon R, Wahrendorf J Diet, bowel function, fecal characteristics and large bowel cancer in Denmark and Finland Nutr Cancer 1982, 4 5-19

28 Domellof L, Darby L, Hanson D, Mathews L, Simi B, Reddy BS Fecal sterols and bacterial β-glucuronidase activity a preliminary metabolic epidemiology study of healthy volunteers from Umea, Sweden and Metropolitan New York Nutr Cancer 1982, 4 120-7
29 Fnstrom J Colorectal cancer and consumption of beef and fat U Br J Cancer 1975, 32 432-9
30 McMichael AJ, Potter JD, Hetzel BS Time trends in colorectal cancer mortality in relation to food and alcohol consuption United States, United Kingdom, Australia and New Zealand Int J Epidemiol 1979, 8 295-303
31 Jain M, Cook GM, Davis FG, Grace MG, Howe GR, Miller AB A case control study of diet and colorectal cancer Int J Cancer 1980, 26 757-68
32 Higginson J Etiological factors in gastrointestinal cancer U J Natl Cancer Inst 1966, 37 527-45
33 Wynder EL, Shigematsu T Fnvironmental factors of cancer of the colon and rectum Cancer 1967, 20 1520 61
34 I A R C Intestinal Microecology Group Dietary fibre, transit time, faecal bacteria, steroids and colon cancer in two Scandinavian populations Lancet 1977, ii 207-11
35 Bjelke E Case-control study of cancer of the stomach, colon and rectum In Oncology 1970 Proceedings of the tenth international cancer congress, vol 5, Chigago, Year Book Med Publ 1971, 320 34
36 Dales LG, Friedman GD, Ury HK, Grossman S, Williams SR A case-control study of relationships of diet and other traits to colorectal cancer in American Blacks Am J Epidemiol 1979, 109 132-44
37 Modan B, Barrell V, Lubin F, Modan M Dietary factors and cancer in Israel Cancer Res 1975, 35 3503-06
38 Kelsey JL A review of research on effects of fiber intake on man Am J Clin Nutr 1978, 31 142-59
39 Story JA Dietary fiber and lipid metabolism Proc Soc Exp Biol Med 1985, 180 447 52
40 Story JA, Kritchevsky D Bile acid metabolism and fiber Am J Clin Nutr 1978, 31 S199-202
41 Bingham S, Williams DR, Cole TJ, James WPT Dietary fibre and regional large bowel cancer mortality in Britain Br J Cancer 1979, 40 456-63
42 Nigro ND, Singh DV, Campbell RL, Pak MS Effect of dietary beef fat on intestinal tumor formation by azoxymethane in rats J Natl Cancer Inst 1975, 54 439 42
43 Reddy BS, Weisburger JH, Wynder EI Effects of dietary fat level and dimethylhydrazine on fecal acid and neutral sterol excretion and colon carcinogenesis in rats J Natl Cancer Inst 1974, 52 507-11
44 Narisawa T, Magadia NE, Weisburger JH, Wynder FL Promoting effect of bile acids on colon carcinogenesis after intrarectal instillation of N-methyl-N' nitro-N-nitrosoguanidine in rats J Natl Cancer Inst 1974, 53 1093 7
45 Reddy BS, Narisawa R, Weisburger JH, Wynder EL Promoting effect of sodium deoxycholate on colon adenocarcinomas in germfree rats J Natl Cancer Inst 1976, 56 441 2
46 Reddy BS, Watanabe K, Weisburger JH, Wynder EI Promoting effect of bile acids in colon carcinogenesis in germ free and conventional F 344 rats Cancer Res 1977, 37 3238 42
47 Raicht RF, Cohen BI, Fazzini E, Sarwal A, Takahashi M Effects of bile acids on induced colon cancer in rats Gastroenterology 1978, 75 981
48 Fleiszer P, Murray D, MacFarlane J, Brown RA Protective effect of dietary fibre against chemically induced bowel tumours in rats Lancet 1978, ii 552 3
49 Freeman HJ, Spiller GA, Kim YS A double blind study on the effects of differing cellulose and pectin fibre diets on 1,2 dimethylhydrazine induced rat colonic neoplasia Cancer Res 1980, 40 2661-5
50 Castleden WM Prolonged survival and decrease in intestinal tumours in dimethylhydrazine treated rats fed a chemically defined diet Br J Cancer 1977, 35 491-5

51 Silverman SJ, Andrews AW Bile acids Comutagenic activity in the Salmonella-Mamma lian-Mmicrosome Mutagenity test Brief communication J Natl Cancer Inst 1977, 59 1557-9
52 Wilpart M, Mainguet P, Maskens A, Roberfroid M Mutagenity of 1,2 dimethylhydrazine towards Salmonella typhimurium, co-mutagenic effect of secondary bile acids Carcinogenesis 1983, 4 45 8
53 Wilpart M, Mainguet P, Maskens A, Roberfroid M Structure-activity relationship amongst biliary acids showing co mutagenic activity towards 1,2-dimethylhydrazine Carcinogenesis 1983, 4 1239 41
54 Kulkarni MS, Heidepriem PM, Yielding KI Production by lithocholic acid of DNA strand breaks in L1210 cells Cancer Res 1980, 40 2666-9
55 Kelsey MI, Pienta RJ Transformation of hamster embryo cells by cholesterol-α epoxide and lithocholic acid Cancer Lett 1979, 6 143 9
56 Reddy BS, Sharma C, Simi B, Fngle A, Laakso K, Puska P, Korpela R Metabolic epidemio logy of colon cancer effect of dietary fiber on fecal mutagens and bile acids in healthy subjects Cancer Res 1987, 47 644-8
57 Deschner EE Relationship of altered cell proliferation to colonic neoplasia In Malt RA, Williamson RCN, eds Colonic carcinogenesis MTP Press Limited, Lancaster Boston The Hague 1982 25-8
58 Deschner EE, Lipkin M, Solomon C In vitro study of human epithelial cells II H3 thymi dine incorporation into polyps and adjacent mucosa J Natl Cancer Inst 1966, 36 849-57
59 Deschner EF, Raicht RF Kinetic and morphologic alterations in the colon of a patient with multiple polyposis Cancer 1981, 47 2440 5
60 Roshin J, Wilson PS, Bull AW, Nigro ND Ornithine decarboxylase activity in the rat and human colon Cancer Res 1984, 44 3226 30
61 Pegg AF, McCann PP Polyamine metabolism and function Am J Physiol 1982, 243 C212-21
62 Luk GD, Baylin SB Polyamines and intestinal growth-increased polyamine biosynthesis after jejunectomy Am J Physiol 1983, 245 G656-60
63 Deschner EE, Raicht RF Influence of bile on kinetic behaviour of colonic epithelial cells Digestion 1979, 19 322 7
64 Bagheni SA, Bolt MG, Boyer JL, Polman RH Stimulation of thymidine incorporation in mouse liver and biliary tract epithelium by lithocholate and deoxycholate Gastroenterology 1978, 74 188 92
65 Deschner EE, Cohen RI, Raicht RF Acute and chronic effect of dietary cholic acid on colo nic epithelial cell proliferation Digestion 1981, 21 290 6
66 Takamo S, Matsushima M, Erturk F, Bryan GT Early induction of rat colonic epithelial ornithine and S-adenosyl-Lmethionine decarboxylase activities by N-methyl-N'-nitro N nitrosoguanidine or bile salts Cancer Res 1981, 41 624-8
67 Narisawa T, Hosaka S, Niwa M Prostaglandin E2 counteracts the inhibition by indometha-cin of rat colon ornithine decarboxylase induction by deoxycholic acid Jpn J Cancer Res 1985, 76 338 44
68 De Rubertis FR, Craven PA, Saito R Bile salt stimulation of colonic epithelial proliferation Evidence for involvement of lipoxygenase products J Clin Invest 1984, 74 1614-24
69 Bruce WR Recent hypotheses for the origin of colon cancer Cancer Res 1987, 47 4237-42
70 Rafter JJ, Eng VWS, Furrer R, Medline A, Bruce WR Effects of calcium and pH on the mucosal damage produced by deoxycholic acid in the rat colon Gut 1986, 27 1320 9
71 Wargovich MJ, Eng VWS, Newmark HI , Bruce WR Calcium ameliorates the toxic effect of deoxycholic acid on colonic epithelium Carcinogenesis 1983, 4 1205-7
72 Lipkin M, Newmark H Effect of added dietary calcium on colonic epithelial-cell prolifera-tion in subjects at high risk for familial colonic cancer N Engl J Med 1985, 313 1381-4

73 Hill MJ, Drasar BS, Williams RFO, Meader TW, Cox AG, Simpson JEP, Morson BC Faecal bile acids and clostridia in patients with cancer of the large bowel. Lancet 1975; i: 535-9.
74. Reddy BS, Wynder EL. Metabolic epidemiology of colon cancer Fecal bile acids and neutral steroids in colon cancer patients and patients with adenomatous polyps. Cancer 1977; 39: 2533-9.
75. Moskovitz M, White C, Barnett RN, Stevens S, Russel E, Vargo D, Floch MH. Diet, fecal bile acids, and neutral sterols in carcinoma of the colon. Dig Dis Sci 1979; 24: 746-51.
76. Mudd DG, McKelvey STD, Norwood W, Elmore DT, Roy AD. Faecal bile acid concentrations of patients with carcinoma or increased risk of carcinoma in the large bowel. Gut 1980; 21: 587-90.
77. Kaibara N, Sasaki T, Ikeguchi M, Koga S, Ikawa S. Fecal bile acids and neutral sterols in Japanese with large bowel carcinoma Oncology 1983; 40: 255-8.
78. Tanida N, Hikasa Y, Shimoyama T, Setchell KDR. Comparison of faecal bile acid profiles between patients with adenomatous polyps of the large bowel and healthy subjects. Gut 1984; 25: 824-32.
79. Hikasa Y, Tanida N, Ohno T, Shimoyama T Faecal bile acid profiles in patients with large bowel cancer in Japan. Gut 1984; 25: 833-8.
80. Breuer NF, Domes P, Jackel S, Goebell H. Fecal bile acid excretion pattern in colonic cancer patients. Dig Dis Sci 1985; 30: 852-9.
81. Murray WR, Blackwood A, Trotter JM, Calman KC, MacKay C. Faecal bile acids and clostridia in the aetiology of colorectal cancer. Br J Cancer 1980; 41: 923-8.
82. Nagengast FM, Werf van der SDJ, Lamers HLM, Hectors MPC, Buys WCAM, Tongeren van JHM. Influence of age, intestinal transit time, and dietary composition on fecal bile acid profiles in healthy subjects. Dig Dis Sci 1988; 33: 673-8.
83. Werf van de SDJ, Huybregts AWM, Lamers HLM, Berge Henegouwen van GP, Tongeren van JHM. Age dependent differences in human bile acid metabolism and 7α-dehydroxylation. Eur J Clin Invest 1981; 11: 425-431.
84. Werf van der SDJ, Nagengast FM, Berge Henegouwen van GP, Huybregts AWM, Tongeren van JHM. Colonic absorption of secondary bile-acids in patients with adenomatous polyps and in matched controls Lancet 1982; i: 759-62.
85. Werf van der SDJ, Nagengast FM, Berge Henegouwen van GP, Huybregts AWM, Tongeren van JHM Intra colonic environment and the presence of colonic adenomas in man. Gut 1983; 24: 876-80.
86. Moorehead RJ, Campbell GR, Donaldson JP, McKelvey STD. Relationship between duodenal file acids and colorectal neoplasia. Gut 1987, 28, 1454-9.
87. Owen RW, Dodo M, Thompson MH, Hill MJ. Fecal steroids and colorectal cancer. Nutr Cancer 1987; 9: 73-80.
88. Arnesjo B, Bodwall B, Stahl E. Bile acid pattern of gallbladder and hepatic bile in patients with and without cholesterol gallstones. Acta Chir Scand 1975; 141: 135-8.
89. Fisher MM, Yousef IM. Sex differences in the bile acid composition of human bile: studies in patients with and without gallstones. Can Med Assoc J 1973; 109: 190-3.
90. Heuman R, Norrby S, Sjodahl R, Tiselius HG, Tagesson C. Altered gallbladder bile composition in gallstone disease. Scand J Gastroenterol 1980; 15: 581-6.
91. Linden van der W. Bile acid pattern of patients with and without gallstones. Gastroenterology 1971; 60: 1144-5.
92 Werner B, Heer de K, Mitschke H. Cholecystektomie und experimentell erzeugtes Dickdarmkarzinoom. I angenbecks Arch Chir 1977; 343: 267-74
93. Schattenkerk ME, Li AKC, Jeppsson BW, Eggink WP, Jamieson CG, Ross JS, Malt RA. Cholecystectomy has no influence on frequency of chemically induced colonic cancer in mice. Br J Cancer 1980, 42: 791-3

94. McMichael AJ, Potter JD. Host factors in carcinogenesis: certain bile-acid metabolic profiles that selectively increase the risk of proximal colon cancer. J Natl Cancer Inst 1985; 75: 185-91.
95. Linos DA, O'Fallon WM, Beart RW jr, Dockerty MB, Kurland LT. Cholecystectomy/and cancer of the colon. Lancet 1981; ii: 379-81
96. Adami HO, Meirik O, Gustavsson S, Nyren O, Krusemo UB. Colorectal cancer after cholecystectomy: Absence of risk increase within 11-14 years. Gastroenterology 1983; 85: 859-65.
97. Berge Henegouwen van GP, Werf van der SDJ, Ruben ATh. Effect of long term lactulose ingestion on secondary bile salt metabolism in man: potential protective effect of lactulose in colonic carcinogenesis. Gut 1987; 28: 675-80.
98. Nagengast FM, Hectors MPC, Buys WCAM, Tongeren van JHM Inhibition of secondary bile acid formation in the large intestine by lactulose in healthy subjects of two different age groups. Eur J Clin Invest 1988; 18: 56-61.

CHAPTER II

Determination of individual human faecal bile acids by gas-liquid chromatography after enzymatic deconjugation and simultaneous solvolysis and methylation using dimethoxypropane

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CHAPTER II

Determination of individual human faecal bile acids by gas-liquid chromatography after enzymatic deconjugation and simultaneous solvolysis and methylation using dimethoxypropane

Abstract

A simple procedure for the determination of bile acids in freeze-dried faeces is described. After enzymatic deconjugation and saponification, bile acids are simultaneously solvolysed and methylated using 2,2 dimethoxypropane. Analysis of the individual bile acids is performed by gas-liquid chromatography. Recoveries of (un)conjugated and sulfated bile acids, added to faeces, were adequate and reproducible. The extraction efficiency for 2 endogenously present bile acids, investigated by injecting radiolabelled bile acids in two patients, proved to be satisfactory.
The advantage of the proposed method is the rapid determination of bile acids in a considerable number of faecal samples, allowing the measurement of the major human faecal bile acids including conjugates and sulfates.

Introduction

In recent years there has been a renewed interest in the measurement of faecal bile acids, because of the possible involvement in colorectal carcinogenesis [1, 2].
Several methods have been employed for the determination of individual bile acids in human faeces [3-8]. Major problems in the measurement are the extraction of bile acids from the particulate matter in the stool and interference of fatty acids and neutral steroids in the gas-liquid chromatographic (GLC) analysis.
Furthermore, conjugated and/or sulfated bile acids can be present in human faeces; therefore a deconjugation and solvolysis step should be included in the analytical procedure [9-15].
Recently, a very detailed method has been described in which all faecal bile acid metabolites are measured, including conjugated and sulfated [16]. The procedure,

however, is time consuming and needs expensive and not widely available equipment like capillary GLC-mass spectrometry.
We developed a rapid gas-liquid chromatographic procedure for the determination of individual human faecal bile acids, using enzymatic deconjugation and subsequently solvolysis and methylation in one step with 2.2-dimethoxypropane.

Materials and methods

Chemicals
All reagents for extraction and analysis were of analytical grade and obtained from Merck Darmstad (FRG). β-Cholylglycine hydrolase (EC 35.12.24; sp. act. 7194 U/mg protein; 1.39 mg protein/ml) was obtained from Schwarz-Mann (Orangeburg, NY, USA).

Bile acid standards
The following reference bile acids were obtained from Steraloids Inc. (Wilton, NH, USA): lithocholic acid (LCA), deoxycholic acid (DCA), chenodeoxycholic acid (CDCA), cholic acid (CA) and 7-ketodeoxycholic acid (7-K-DCA). Taurocholic acid (TCA) and glycocholic acid (GC) were obtained from Calbiochemical (San Diego, CA, USA). The sulfated bile acids 3α-sulfolithocholic acid (SLCA), 3α-sulfodeoxycholic acid (SDCA) and sulfochenodeoxycholic acid (SCDCA; consisting of 70% 3α-sulfo CDCA and 30% 7α-sulfo CDCA) were obtained from P.L.-Biochemicals (Milwaukee, WI, USA). All reference bile acids were more than 95% pure.
Radiolabelled [24-^{14}C] lithocholic acid (50 mCi/mmol) and [24-^{14}C] deoxycholic acid (60 mCi/mmol) were obtained from the Radiochemical Centre, Amersham (UK). [24-^{14}C] Chenodeoxycholic acid (50 mCi/mmol) was obtained from New England Nuclear (Boston, MA, USA).

Instruments
Faeces were homogenized using a Philips mixer and freeze-dried subsequently in a Breda Scientific Freeze dryer LY-S-FM. Gas-liquid chromatography was performed on a F&M 402 high efficiency chromatograph (Hewlett Packard, Avondale, USA) with a U-shaped all glass column, 1.20 m x 3 mm i.d., packed with 1% OV-210 on Gaschrom Q, 100-120 mesh (Chrompack, Middelburg, The Netherlands). Operating conditions for gas liquid chromatography were: oven temperature 235 °C, temperature injection point 250 °C, temperature flame ionisation detector 245 °C. Nitrogen was used as a carrier gas, flow rate 20 ml/min; hydrogen 40 ml/min, air 310 ml/min. Retention times and peak areas were measured with a computing integrator (Minigrator, Spectra-Physics, Eindhoven, The

Netherlands) and chromatograms recorded on a recorder of Honeywell Controls LTD (UK) Radioactivity was determined in a Philips liquid scintillation counter PW4700 (Philips, The Netherlands). Combustion of faecal samples for determination of total ^{14}C-radioactivity was performed in a sample oxidator, according to Griffith [17].

Collection and preparation of faeces
Fresh voided faeces were collected and frozen in solid CO2. After weighing it was diluted with a known amount of distilled water and homogenized with a mixer at a temperature of 80 °C (to speed up thawing and denaturate bile acid converting enzymes) A sample (10 g) was immediately frozen (−20 °C), lyophilized during 48 hours and stored at −20 °C until analysis.

Analytical procedure
The method is a modification of the Grundy technique [3] The procedure is illustrated in the flow sheet (figure 1) The procedure is described here stepwise:

Enzymatic deconjugation
50 mg lyophilized faeces are weighed in a glass stoppered centrifuge tube. Two millilitres of 0 1 mol/l sodium acetate buffer (pH 5.7 with 2 0 gram EDTA liter ^{1} and 0 2 ml β-mercaptoethanol liter ^{1}) and 50 μl cholylglycine hydrolase are added Incubation is carried out overnight in a waterbath at 37 °C.

Saponification
To each sample 0,1 ml 5 mmol/l 7-K-DCA is added as an internal standard to correct for losses during the procedure. One ml 10 mol/l sodium hydroxide, 6 ml methanol and some boiling chips are added and the mixture is refluxed for 2 hours at boiling point

Extraction of neutral steroids
Neutral steroids are extracted 3 times in petroleum ether after shaking and centrifugation.

Solvolysis and methylation
After the extraction of the neutral steroids 1,0 ml HCL and per ml aqueous solution 7,0 ml DMP is added After mixing, incubation is carried out for 18 hours in the dark (to prevent formation of pigment polymers) at 37 °C. After this overnight incubation the mixture is evaporated under a stream of nitrogen at 50 °C. Beyond this temperature a dark brown residu will appear due to the formation of pigment polymers.

Extraction of bile acids.
7 ml aqua dest is added to the residu and the methylated bile acids are extracted 3 times with 10 ml diethylether. After completion the diethylether is evaporated under a stream of nitrogen.

Derivatisation.
The methylated bile acids are converted into their trifluoroacetates by addition of 0.2 ml trifluoroacetic anhydride to the residu and incubation in closed tubes for 20 minutes at 37 °C.

Gas-liquid chromatography.
The trifluoroacetic anhydride is evaporated and the residu dissolved in 0,2 ml acetone. After injecting a 1 μl sample in the gaschromatograph the bile acids are identified by comparing the retention times to those of reference bile acids and for one keto bile acid with a reference in the literature on a QF-1 column. The amount of bile acid in each sample is calculated by dividing the peak area of the bile acid by that of the internal standard, multiplied by the amount of internal standard added to the sample and corrected for the response factor of the flame ionizing detector.

Liquid scintillation counting.
In case recovery experiments with radio-labeled bile acids are performed, 0.1 ml of the final acetone mixture is transferred into a counting vial and evaporated. The residu is dissolved in a PPO/POPOP mixture (5:0.2/liter) in toluene and counted with external standardization for quench correction.

Determination of accuracy and precision.
The accuracy of the method is determined by measuring the recoveries of added reference bile acids to lyophilized faeces. Furthermore, the recovery of endogenously present DCA and CDCA in the stool of two patients (injected with ^{14}C-labelled DCA and CDCA for kinetic studies) is determined by comparing radioactivity in the final acetone extracts with radioactivity measured after direct combustion of the faecal samples.
The intra-assay precision is evaluated by calculating the coefficients of variation from the duplicate measurements of LCA and DCA in a control sample on different days. The inter-assay precision is calculated from the means of the duplicate measurements each day.

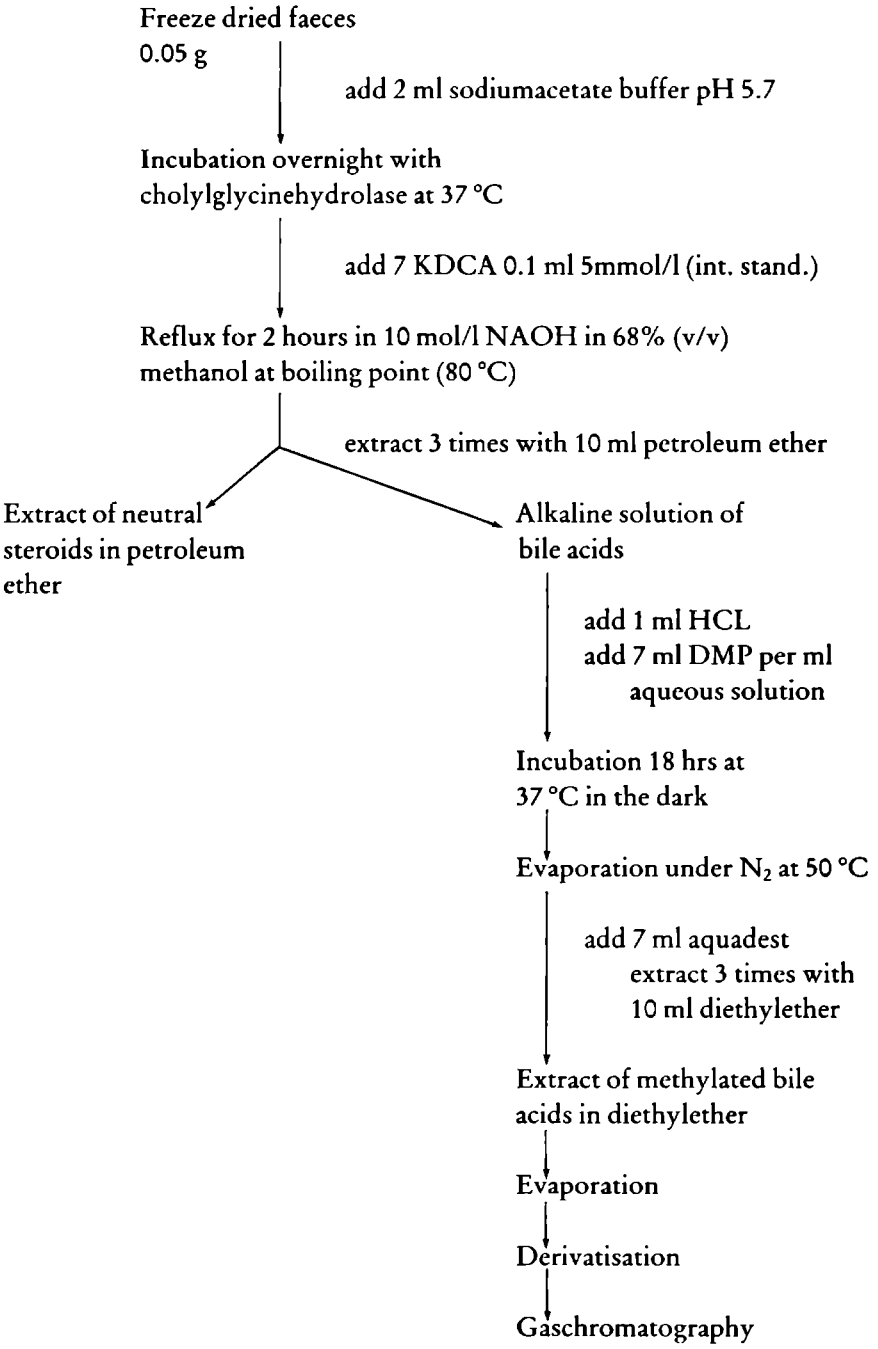

Figure 1 Flow sheet of the method for the analysis of human faecal bile acids.

Results

In figure 2A, a gas-liquid chromatogram of a faecal sample with secondary bile acids is shown. Figure 2B shows a gas-liquid chromatogram of a faecal sample in which a considerable quantity of primary bile acids are present. A reasonable separation of bile acids is achieved within 10 minutes. Unfortunately on an OV-210 column isolithocholic acid (3β-hydroxy-5β-cholanic acid) can not be separated from lithocholic acid. In contrast, isodeoxycholic acid (3β, 12α dihydroxy-5β-cholanic acid) can be separated from deoxycholic acid. At the end of the run keto bile acids are eluted, of which 3β, 12 keto-5β-cholanic acid and 3α, 12 keto-

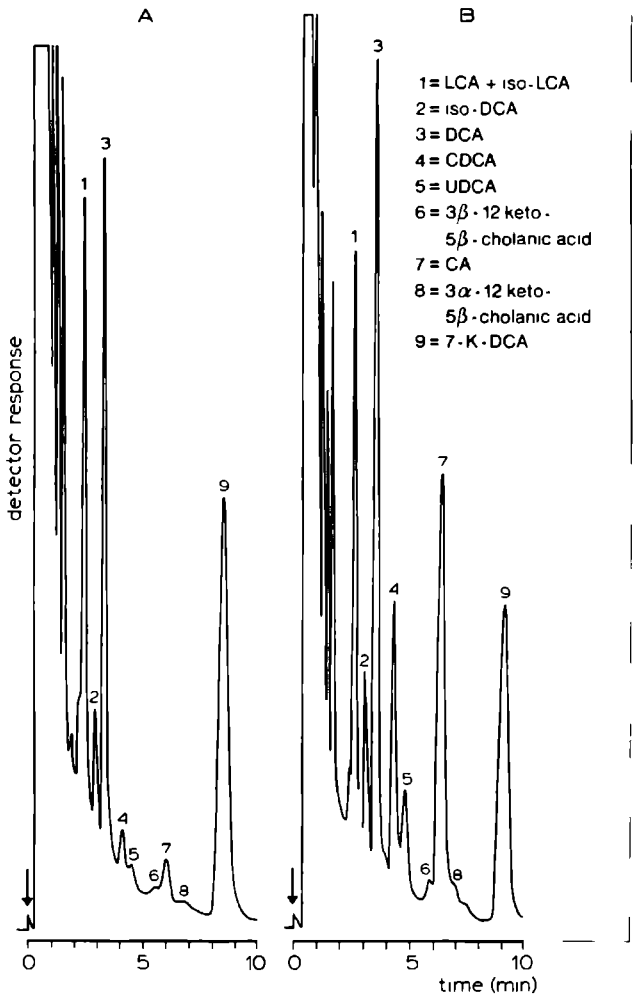

Figure 2 Gas-liquid chromatogram of faecal bile acids in two different stools. A: with secondary bile acids and a minor fraction of primary bile acids. B: with secondary and primary bile acids.

Table I Recovery of radiolabelled reference bile acids added to faeces

No. of samples	Added bile acid	Recovery (% ± SD)
10	[24-^{14}C] LCA	89.7 (±1.6)
12	[24-^{14}C] DCA	86.3 (±1.7)
10	[24-^{14}C] CDCA	85.8 (±2.6)

Table II Recovery of reference bile acids added to faeces, determined by gas-liquid chromatography

No. of samples	Added bile acid	Recovery (% ± SD)
8	LCA	95.8 (± 5.0)
8	DCA	95.5 (± 6.5)
8	CDCA	96.2 (± 8.2)
4	CA	92.8 (± 4.0)
4	GCA	85.5 (± 0.7)
4	TCA	84.5 (± 7.7)
8	SLCA	100.0 (±10.2)
8	SDCA	101.3 (± 3.0)
8	SCDCA	98.0 (± 5.4)

5β-cholanic acid can be identified. However, these keto bile acids are not always completely separated from cholic acid.

Recoveries of added radiolabelled reference bile acids are shown in table I and proved to be sufficient and reproducible. Recoveries of two conjugated, four unconjugated and three sulfated bile acids, measured by GLC, are sufficient and reproducible as is shown in table II. Recoveries of two endogeneous bile acids (DCA and CDCA) were determined in two patients. In one patient (injected with [24-^{14}C]DCA) only secondary bile acids were present in the stool; in the other patient (injected with [24-^{14}C]CDCA) only primary bile acids were detected in the stool. Faeces was collected during four days. Combustion of faeces in a sample oxidator (±1 gram wet faeces) was used as a reference. The efficacy of the combustion had been determined by adding a known amount of [24- ^{14}C] DCA to wet fresh voided faeces. The recovery of the $^{14}CO_2$ was 96.7±6.9% (n=5) of the calculated radioactivity. After extraction of the faeces of the two patients, the

recovery of DCA was 84.2±3.9% (mean±SD) and of CDCA 87.9±7.3% (mean±SD), compared to combustion in the sample oxidator, as is shown in table III. The intra-assay precision for the determination of the most prominent faecal bile acids LCA and DCA was 2.8% and 4% respectively. The inter-assay precision was 9.0% and 7.2% for LCA and DCA respectively.

Table III Recovery of ^{14}C from faeces on 4 consecutive days[a].

Patient 1[b] [24-^{14}C]DCA	Radioactivity after combustion (disintegrations g^{-1} faeces)	Radioactivity after extraction (disintegrations g^{-1} faeces)	Recovery %
Day 1	1220	1040	85.2
Day 2	8455	7336	86.7
Day 3	6477	5601	86.5
Day 4	10525	8257	78.4
Patient 2[c] [24-^{14}C]CDCA			
Day 1	8419	7441	88.4
Day 2	16193	12891	79.6
Day 3	7077	6890	97.4
Day 4	5076	4383	86.3

a After intravenous injection of [24-^{14}] DCA or [24-^{14}C] CDCA in two patients
b Patient with malignant adenoma of the colon and secondary bile acids in faeces.
c Patient with bile acid induced diarrhoea and only primary bile acids in faeces

Discussion

Most extraction procedures of faecal bile acids are complex, use several purification steps and are time consuming. The Grundy method [3], which is regarded as our reference procedure does not allow the measurement of sulfated bile acids and some keto bile acids. Furthermore, the strong alkaline hydrolysis which is used, can give rise to artefacts [18], like the formation of unsaturated derivates of sulfated chenodeoxycholic acid. In our method alkaline hydrolysis is replaced by enzymatic deconjugation. The conjugated bile acids are hydrolysed sufficiently.

Bile acids are extracted from the particulate matter by refluxing in an alkaline methanolic solution. We use methanol instead of ethanol to prevent the formation of bile acid ethylesters. The extraction of endogenous DCA and CDCA is acceptable by refluxing for two hours. The extraction efficiency of endogenous LCA could not be detected unfortunately. However, Podesta et al extracted 99% of the radioactivity in faeces of a healthy volunteer who ingested [24-^{14}C] CDCA by refluxing two hours in an alkaline ethanolic solution [9]. Since CDCA is normally almost completely dehydroxylated to LCA (+ isoLCA) in the large bowel, the radioactivity would be present in this last fraction.

After the saponification step neutral steroids must be extracted with petroleum ether, because coprostanon can interfere with the gas liquid chromatographic analysis on OV-210. This compound is eluted at the same time as (iso) lithocholic acid.

The extent of sulfation of bile acids in human faeces is still debated. Some investigators found up to 40% sulfated bile acids in human faeces [9, 10], whereas, others found only a minor fraction (less than 5%) sulfated bile acids in human stool [11, 12, 14, 15]. Because sulfated bile acids (mainly sulfated lithocholic acid) can be present in human faeces we decided to carry out a solvolysis procedure.

The solvolysis procedure originally described by Javitt [19] and applied to serum by Cantafora et al [20] seemed appropriate to use for faecal bile acids. An advantage of the method is the simultaneous methylation of the free bile acids that occurs in the acidified anhydrous mixture [21]. To split CDCA-7-sulfate incubation overnight at 37 °C is warranted [22]. We have tried to measure the amount of unsulfated bile acids by extraction of the bile acids after saponification with diethylether at an acidic pH. However, in accordance with Pageaux et al [23] added sulfolithocholic acid was partly solvolysed during this procedure. Even when the extraction was performed at a lower temperature or higher pH partial solvolysis took place. So, quantitative measurement of unsulfated bile acids was not possible.

Recently a rapid extraction procedure for the determination of faecal bile acids was published [8]. Recoveries, however, were measured qualitatively using thin-layer chromatography or quantitatively by enzymatic determination.

In conclusion, the proposed method for the determination of the major bile acid metabolites in human stool by gas liquid-chromatography is rapid and does allow the determination of a considerable number (about 48) of faecal samples in 3 days. The simultaneous solvolysis and methylation of the bile acids after saponification is of particular advantage. Conjugated and sulfated bile acids which may be present in human faeces are included in the determination of the individual bile acids.

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References

1 Hill MJ, Drasar BS, Williams REO et al Faecal bile acids and clostridia in patients with cancer of the large bowel Lancet 1975, i 535-39
2 Cohen BI, Raicht RF, Deschner EE et al Effect of cholic acid feeding an N-methyl N nitroso mean induced colon tumours and cell kinetics in rats J Natl Cancer Inst 1980, 64 573-78
3 Grundy SM, Ahrens JrEH, Miettinen TA Quantitative isolation and gas-liquid chromato graphic analysis of total fecal bile acids J Lipid Res 1965, 6 397-410
4 Eneroth P, Gordon B, Ryhage R, Sjovall J Identification of mono- and dihydroxy bile acids in human feces by gas-liquid chromatography and mass spectrometry J Lipid Res 1966, 7 511-23
5 Eneroth P, Gordon B, Sjovall J Characterization of trisubstituted cholanic acids in human feces J Lipid Res 1966, 7 524-30
6 Evrard E and Janssen G Gas-liquid chromatographic determination of human fecal bile acids J Lipid Res 1968, 9 226-36
7 Roseleur OJ, van Gent CM Alkaline and enzymatic hydrolysis of conjugated bile acids Clin Chim Acta 1976, 66 269-72
8 Van den Ende A, Radecker CE, Mairnhu WM, Van Zanten AP Improved extraction procedure for determination of bile acids in faeces Clin Chim Acta 1982, 121 95 109
9 Podesta MT, Murphy GM, Dowling RH Measurement of faecal bile acid sulphates J Chromatogr 1980, 182 293-300
10 Salvioli G, Salati R Faecal bile acid loss and bile acid pool size during short term treatment with ursodeoxycholic and chenodeoxycholic acid in patients with radiolucent gallstones Gut 1979, 20 698-704
11 Islam MA, Raicht RI, Cohen BI Isolation and quantitation of sulfated and unsulfated ste roids in human feces Anal Biochem 1981, 112 371-77
12 Mc Junckin B, Fromm R, Sarva P, Amin P Factors in the mechanism of diarrhea in bile acid malabsorption faecal pH, a key determinant Gastroenterology 1981, 80 1454-64
13 Van Faassen A, Huijbregts AWM Letter to the editor J of Lipid Res 1982, 23 508
14 Tanida N, Hikase Y, Shimoyaura T, Setchell KDR Comparison of faecal bile acid profiles between patients with adenomatous polyps of the large bowel and healthy subjects in Japan Gut 1984, 25 824-32
15 Hikasa Y, Tanida N, Ohno T, Shimoyana T Faecal bile acid profiles in patients with large bowel cancer in Japan Gut 1984, 25 832 8
16 Setchell KDR, Lawson AM, Tanida N, Sjovall J General methods for the analysis of metabolic profiles of bile acids and related compounds in feces J of Lipid Res 1983, 24 1085 1100
17 Griffith MH, Mallinson A A furnace for combustion of biological material containing tritium and carbon-14 labeled compounds Anal Biochem 1968, 2 465-73
18 Lepage G, Fontaine A, Roy CC Vulnerability of keto bile acids to alkaline hydrolysis J Lipid Res 1978, 19 505 09

19 Javitt NB, Lavy U Serum chenodeoxycholate in neonatal liver disease 1974 Advances in bile acid research (Matern S, Hackenschmidt J, Back P, Gerok eds) p 123 FK Schattauer Verlag, Stuttgart New York
20 Cantafora A, Angelico M, Attili AF, Ersoli E, Copocaccia E An improved gas-chromatographic method for the determination of sulfated and unsulfated bile acids in serum Clin Chim Acta 1979, 95 501-8
21 Shaw R, Elliott WH Bile acids LV 2,2-Dimethoxypropane an esterifying agent preferred to diazomethane for chenodeoxycholic acid J Lipid Res 1978, 19 783-7
22 Cohen BI, Budai K, Javitt NB Solvolysis of chenodeoxycholic acid sulfates Steroids 1981, 37 621-26
23 Pageaux JF, Duperray B, Anker D, Dubois M Bile acid sulfates in serum bile acids determination Steroids 1978, 34 73 88

CHAPTER III

Influence of age, intestinal transit time and dietary composition on fecal bile acid profiles in healthy subjects

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CHAPTER III

Influence of age, intestinal transit time and dietary composition on fecal bile acid profiles in healthy subjects

Abstract

Bacterial transformation of bile acids is possibly involved in colorectal carcinogenesis. In several epidemiological studies the secondary bile acid concentration in feces is related to the incidence of colonic cancer. However, data on fecal bile acids in case-control studies are conflicting. We investigated the influence of age, intestinal transit time and dietary composition on fecal bile acid profiles in healthy subjects of 3 different age groups (mean age 22, 48 and 67 years). Fecal bile acids were analysed by gas-liquid chromatography.

The concentration of the major secondary fecal bile acids increased with advancing age and was significantly higher in elderly subjects, compared to young adults. The concentration in middle-aged persons was intermediate. Analysis of dietary constituents showed that the fat intake in the three groups was comparable. The dietary fiber intake in elderly subjects was significantly lower than in both other groups. The former group did excrete less dry fecal material compared to both other groups. Dietary fiber intake was negatively correlated with the total bile acid concentration. Probably, a decrease in dietary fiber intake results in higher fecal bile acid concentrations with advancing age.

From the findings of this study it is obvious that matching for age is important when case-control studies concerning the role of fecal bile acids in colorectal carcinogenesis are conducted.

Introduction

Bacterial transformation of bile acids in the large bowel has been implicated in colorectal carcinogenesis [1]. Dietary habits can influence bile acid metabolism [2]. A high animal fat intake increases fecal bile acid concentration and excretion [3, 4] and is positively correlated with the incidence of colorectal cancer [5, 6]. A high dietary fiber intake may protect against this malignancy by accelerating intestinal transit, diluting colonic contents, binding bile acids and lowering colonic pH[7-12]. Elevated fecal bile acid concentrations in patients with large bowel

cancer or lesions predisposing to this tumour (e.g. adenomas) have been found by some [13, 14], but not all investigators [15-21]. Reasons for the discrepancy could be different selection procedures for both patients and controls, as well as differences in age, intestinal transit time and dietary composition between patients and controls. To gain more insight into these relationships we studied the influence of the latter parameters on fecal bile acid profiles in 34 healthy subjects.

Materials and methods

Subjects

Detailed data of all participating healthy volunteers of three different age groups with a mean age of 22, 48 and 67 years are presented in table 1. Subjects who had undergone cholecystectomy or major intestinal surgery, those with known liver, gallbladder or intestinal disease or suffering from diarrhoea, hyperlipoproteinaemia or diabetes mellitus and persons using drugs, especially antibiotics, laxatives and hypnotics were excluded. Alcohol consumption not exceeding about 10 g daily was allowed. Body weight was recorded three times during the study and proved to be stable in all subjects. Physical examination, tests for glucose and protein in the urine and blood analysis for ESR, liver and renal function and fasting serum lipids did reveal only a slightly higher serum cholesterol in middle aged persons compared to young adults. No occult blood and no parasites were

*Table 1 Data on healthy subjects of three different age groups**

	Group A	Group B	Group C	A vs B (P)	B vs C (P)	C vs A (P)
No. of subjects	11	12	11			
Age (years)	21.8 ±1.8	47.6 ±1.4	67.3 ±1.4	<0.01	<0.01	<0.01
Range	21-27	39-53	57-73			
Male/female	5/6	4/8	6/5			
Weight (kg)	67.6 ±1.5	71.7 ±2.6	69.1 ±3.7	ns	ns	ns
△Ideal weight(%)†	−13.0 ±2.4	+ 1.0 ±2.3	+10.8 ±5.2	<0.01	ns	<0.01
Serum cholesterol (mmol/l)	4.58±0.29	5.29±0.17	5.38±0.32	<0.05	ns	ns
Serum triglycerides (mmol/l)	1.03±0.12	1.08±0.09	1.56±0.22	ns	ns	ns

* Values represent mean ± SEM;.ns = not significant

† % of ideal weight = [actual weight (kg/length (cm) − 100] x 100.

detected in the stools of all volunteers. They were not screened however, for colon cancer or adenomas by radiology or endoscopy. Before participation in the study all subjects gave written consent for the study, which was approved by the Human Research Review Committee of our hospital.

Experimental design

None of the subjects was hospitalized during the study. In order to establish the relationship between dietary composition, intestinal transit time and faecal bile acid profiles, all subjects adhered to a standardized diet for the whole study period of 14 days. During the inital visit average food intake was defined from interview data by the same dietician (HL). All participants 4 checked the accuracy of their food intake using written instructions and weighing all food stuffs before consumption. After the necessary adjustments they adhered to this schedule. After keeping this diet for 7 days, stools were collected on day 8 and 9 for fecal bile acid measurements. Intestinal transit time, according to the method of Hinton et al [22] was measured with radiopaque pellets, which were administered on day 10. During another five days stools were collected for radiography to determine the number of pellets excreted. Transit time was calculated from the appearance of 80% of the pellets in the stool.

Composition of the dietary constituents was calculated from Dutch food tables.

Stool collection

All subjects received a container with solid CO_2 (−70 °C) at their homes to collect stools for 2 days. Each of them did produce stools on each of the 2 days. After voiding, feces was immediately frozen in the container to prevent bacterial transformation of the bile acids.

Upon arrival at the laboratory the stools were diluted with a known amount of distilled water and homogenised with a mixer at a temperature of 80 °C to speed up thawing and to inactivate bile acid-converting enzymes. A sample (approx. 10 g) was frozen, lyophilized at −20 °C for 48 hr and stored. Fecal bile acids were measured separately in the two 24-hr specimens.

Fecal bile acid analysis

A detailed description of the fecal bile acid analysis has been published elsewhere [23]. In short the procedure was as follows: Lyophilized feces (50 mg) were incubated overnight at 37 °C in a sodium acetate buffer (pH 5.7) with cholylglycine hydrolase to hydrolyse eventually present conjugated bile acids. Bile acids were subsequently extracted from particulate matter by refluxing in an alkaline milieu in methanol for 2 hr at boiling point.

Before refluxing, 7-ketodeoxycholic acid (7-KDCA) was added as an internal standard in order to correct for losses during the procedure. After extraction of

neutral steroids with petroleumether, the bile acids were solvolysed and methylated with dimethoxypropane The methylated bile acids were extracted with diethylether and converted into their trifluoroacetates Bile acids were identified and quantitated by gas-liquid chromatography (GLC) using a Packard 430 gaschromatograph Bile acids were identified by comparing the retention times to those of reference bile acids The amount of bile acids was determined by dividing the peak area of the bile acid by that of the internal standard (7-KDCA) with correction for the response factor of the flame ionization detector for the individual bile acids. With this method the major fecal bile acids could be separated by GLC satisfactorily. However, isolithocholic acid (iLCA) is not separated from lithocholic acid (LCA) and therefore these bile acids are taken together and referred to as (i)LCA

Statistics

Data of all groups were evaluated with the Wilcoxon's rank sum test with confidence levels at 0 05 or 0 01 Linear regressions have been calculated by the method of least squares; their significance was obtained from Spearman's correlation coefficient r.

Table 2 Dietary constituents and intestinal transit time in healthy subjects of three different age groups

	Group A	Group B	Group C	A vs B (P)	B vs C (P)	C vs A (P)
Caloric intake (kCal day ^{1})	2329±170	1981±110	1842±101	ns	ns	<0 05
Total protein	94±8	82±4	75±5	ns	ns	ns
animal	64±6	60±4	56±4	ns	ns	ns
vegetable	30±3	22±3	19±1	<0 05	ns	<0 05
Total fat	81±8	77±5	79±3	ns	ns	ns
animal	62±8	63±5	64±4	ns	ns	ns
vegetable	19±4	14±3	15±4	ns	ns	ns
Carbohydrates	307±23	236±20	200±14	<0 05	ns	<0 05
Cholesterol	218±20	224±28	210±17	ns	ns	ns
Dietary fiber	37±3	33±3	22±2	ns	<0 05	<0 05
Intestinal transit time	51±9	78±11	46±7	ns	<0 05	ns

Values represent mean ± SEM ns = not significant Dietary constituents in g day ^{1}, cholesterol in mg day ^{1} Intestinal transit time in hours

Results

As is shown in table 1 three groups of healthy subjects of different age groups were studied. The young adults had a significant lower ideal body weight compared to both other groups. In the group middle-aged persons more females were present, otherwise the groups were comparable.
The average daily food intake is shown in table 2. Total caloric intake decreased with advancing age and was significantly lower in elderly persons compared to young adults (-21%). The intake of both vegetable protein and carbohydrates was lower in the middle-aged (-27% and -23%) and elderly persons (-37% and -35%) compared to young adults. Dietary fiber intake was significantly lower in elderly persons compared to both middle-aged and young adults (-33% and -41% respectively).
A remarkable finding was the considerably longer intestinal transit time in the middle-aged persons (table 2) compared to both other groups, which was due to the fact that 4 subjects in this group had an intestinal transit time longer than 120 hr without having constipation subjectively.

*Table 3 Fecal bile acid concentration on two consecutive days in healthy subjects of three different age groups**

	Group A			Group B			Group C		
	day 8	day 9	P	day 8	day 9	P	day 8	day 9	P
(i)Lithocholic acid	4 92±0 58	5 17±0 82	ns	6 73±0 82	6 32±0 84	ns	7.90±0.80	8.85±1 29	ns
Isodeoxycholic acid	0.86±0 25	0 66±0 15	ns	1 09±0 31	1 15±0 84	ns	1 41±0.20	1 49±0.29	ns
Deoxycholic Acid	7 91±0 76	7 58±1 00	ns	8 59±0 94	8 33±0 98	ns	10 77±1 35	11 41±1 87	ns
Total bile acids	15 73±1 22	15 17±1 75	ns	18 74±1 64	18 24±1 84	ns	22 18±1 94	24.03±3 26	ns

* Values respresent mean ± SEM. ns = not significant Fecal bile acid concentration in μmol.g^{-1} dry weight

Fecal bile acid concentrations were measured on two consecutive days and as can be seen in table 3 no significant differences were present for the major bile acids in these separate 24 hr samples in the three groups studied. As no day-to-day variation is present in the measurement of the fecal bile acid concentrations, we concluded that in studies in which the dietary intake is controlled for, reliable data can be obtained by collection and pooling of one- or two-day stool samples.
In table 4 the average concentration for the bile acids over the two days are shown in the sequence of their elution pattern on GLC. The concentration of the major bacterial degraded bile acids steadily increased with advancing age. The concentration of (i)LCA, isoDCA, DCA and 3β-hydroxy-12keto-5β-cholanic acid in elderly persons was significantly higher (+68%, + 73%, +47% and

*Table 4 Average fecal bile acid concentration, fecal weight and dry matter in healthy subjects of three different age groups**

	Group A	Group B	Group C
(i)Lithocholic acid (LCA + iLCA)	4.96±0.65	6.53±0.82	8.38±1.02†
Isodeoxycholic acid	0.84±0.24	1.12±0.31	1.45±0.23†
Deoxycholic acid	7.53±0.78	8.47±0.93	11.10±1.57†
Chenodeoxycholic acid	0.30±0.15	0.12±0.83	0.23±0.07
Ursodeoxycholic acid	0.03±0.02	0.02±0.02	0.05±0.04
3β-OH-12keto-5β-cholanic acid	0.19±0.07	0.61±0.20	0.65±0.13†
Cholic acid	0.50±0.20	0.33±0.13	0.35±0.26
3α-OH-12keto-5β-cholanic acid	0.89±0.13	1.31±0.23	0.93±0.14
Total bile acids	15.25±1.34	18.50±1.71	23.13±2.51†
Fecal weight	144±19	145±20	106±13
Dry matter(%)	24.8±1.5	25.4±2.1	21.4±2.1
Dry fecal material	35.7±1.3	36.8±1.9	22.7±1.2‡

* Values represent mean±SEM. Fecal bile acid concentration in $\mu mol.g^{-1}$ dry weight. Fecal weight and dry fecal material in $g.day^{-1}$

† <0.05 C vs A.

‡ <0.05 C vs A and B.

+242% respectively) compared to young adults. The same applied for the total fecal bile acid concentration(+52%). Concentrations in the middle-aged persons fell between those for young adults and elderly persons, but were not statistically different from these two groups. No data on bile acid excretion are presented, because without correction for fecal flow no reliable figures can be extrapolated from a 2-day stool collection [24].

In the total group of 34 subjects a negative correlation between the dietary fiber intake and the total fecal bile acid concentration ($r=-0,37$; $p<0.05$) was found. No correlation of fat (both animal and vegetable), carbohydrate intake and other dietary constituents with the fecal bile acid concentration was found. Moreover, intestinal transit time was not correlated with the fecal bile acid concentration.

Discussion

Bile acids may play a role in colonic carcinogenesis. In several epidemiological studies the incidence of large bowel cancer is positively related to the fecal bile

acid concentration in the populations studied [30]. Populations with a high risk for the development of this tumor usually did consume more fat (or less fiber) than low-risk populations [29, 30]. Both the influence of a high fat and a low fiber intake could account for this effect. However, results from case-control studies are conflicting [13-21]. Hill et al [13] were the first to show a significant higher fecal bile acid concentration in patients with large bowel cancer compared to patients with other illnesses. These results were confirmed by Reddy and Wynder [14] but since then several investigators have not found these differences [15-21]. Moreover, Murray et al [17] found higher fecal bile acid concentrations in control patients (with minor non-gastrointestinal diseases) than in patients with colorectal cancer.

Several explanations are possible for the observed discrepancies. Differences in the composition of the colorectal cancer patient and control group could play a role. The controls varied from patients with nonmalignant bowel diseases or non-gastrointestinal diseases to healthy subjects. Differences in dietary intake of fat and fiber can, as already mentioned, influence fecal bile acid profiles. No data on dietary composition were stated in any of the case-control studies. A rapid transit of residue through the gut can decrease conversion of bile acids in the colon and increase fecal bile acid output. However, no data on intestinal transit time were given in these studies. Finally, we have shown that 7α-dehydroxylation of cholic acid to deoxycholic acid is age-dependent, suggesting that with increasing age a considerable increase in secondary bile acid formation can occur [31]. Matching for age was not performed in four case-control studies [13-15, 21]. Our results in 3 groups of healthy volunteers of different ages show, that the concentration of the major fecal bile acids increased with advancing age. This increase seems to be the result of differences in dietary constituents among the three groups. As was expected total caloric intake decreased with age. Fat intake (both animal and vegetable) was comparable in the three groups. However, the ratio animal to vegetable fat increased with age. The total protein content did not change, but the ratio of animal to vegetable protein increased. Carbohydrate consumption decreased, which was mainly due to a lower intake of starch. The intake of dietary fiber was significantly lower in elderly persons, compared to both middle-aged and young persons.

The decrease in carbohydrate and fiber consumption, together with the increase in ratios animal to vegetable fat and protein was due to the fact that elderly subjects consumed less vegetable material (e.g. bread, potatoes) than younger adults. The consumption pattern of the middle-aged subjects was between the two other groups. Probably this was the reason that elderly persons excreted significantly less dry faecal material either of the other groups [7, 32]. These factors might well be responsible for their higher fecal bile acid concentrations. The negative correlation between the dietary fiber intake and the total bile acid concentration in all subjects gives support to the important role of fiber in the figures presented.

Despite their lower fiber intake, the intestinal transit time in elderly persons was not longer than in the young adults. The reason for this observation could be the fact, that the fiber intake in the elderly was high enough to maintain a normal colonic function. In this study the intestinal transit time was not a major factor in the fecal bile acid concentration and pattern.

In conclusion, this study shows an age dependent fecal bile acid concentration. A relationship with changing dietary habits with age is possible. No clear relationship with intestinal transit time does exist. These findings should be taken into account when studies concerning fecal bile acid patterns in populations or patients at risk for developing colonic cancer are conducted.

References

1. Hill MJ. Leading article. Bile, bacteria and bowel cancer. Gut 24: 871-875, 1983.
2. Low-Beer TS. Diet and bile acid metabolism Clin Gastroent 6: 165-178, 1977.
3. Cummings JH, Wiggins HS, Jenkins DJA, Houston H, Jivraj T, Drasar BS, Hill MJ. Influence of diets high and low in animal fat on bowel habit, gastrointestinal transit time, fecal microflora, bile acid and fat excretion. J Clin Invest 61. 953-963, 1978.
4. Hill MJ. The effect of some factors on the faecal concentration of acid steroids, neutral steroids and urobilins. J Pathol 104: 239-245, 1971.
5. Reddy BS, Ekelund G, Bohe M, Engle A, Domellof L. Metabolic epidemiology of colon cancer: dietary pattern and fecal sterol concentrations of three populations. Nutr Cancer 5: 34-40, 1983.
6. Reddy BS, Weisburger HJ, Wynder EL. Effects of high risk and low risk diets for colon carcinogenesis on fecal microflora and steroids in man. J Nutr 105: 878-884, 1975.
7. Cummings JH, Hill MJ, Jenkins DJA, Pearson JR, Wiggins HS. Changes in faecal composition and colonic function due to cereal fiber. Am J Clin Nutr. 29: 1468-1473, 1976.
8. Aries VC, Crowther JS, Drasar BS, Hill MJ, Ellis RF. The effect of a strict vegetarian diet on the faecal flora and faecal steroid concentrations. J Pathol 103: 54-56, 1971.
9. Walters RI, McLean Baird L, Davies PS, Hill MJ, Drasar BS, Southgate DAT, Green J, Morgan B Effects of two types of dietary fibre on faecal steroid and lipid excretion. Br Med J 2: 536, 1975.
10. Cummings JH, Hill MJ, Jivraj T, Houston H, Branch WJ, Jenkins DJA. The effect of meat protein and dietary fibre on colonic function and metabolism I. Changes in bowel habit, bile acid excretion and calcium absorption Am J Clin Nutr 32: 2086-2093, 1979.
11 Kay RM, Truswell AS Effect of wheat fibre on gastrointestinal function plasma lipids and steroid excretion in man. Br J Nutr 37: 227-234, 1977.
12. Stasse-Wolthuis M, Albers HFF, Jeveren JGC van, Jong JW de, Hautvast JGAJ, Hermus RJJ, Katan MB, Brydon WG, Eastwood HA.. Influence of dietary fiber from vegetables and fruits, bran or citus pectin on serum lipids, faecal lipids and colonic function. Am J Clin Nutr 33: 1745-1756, 1980.
13. Hill MJ, Drasar BS, Williams REO, Meade TW, Cox AG, Simpson JEP, Morson BC. Faecal bile-acids and clostridia in patients with cancer of the large bowel. Lancet 1: 535-539, 1975.
14 Reddy BS, Wynder EL. Metabolic epidemiology of colon cancer. Faecal bile acids and neutral sterols in colon cancer patients and patients with adenomatous polyps. Cancer 39: 2533-2539, 1977.

15 Moskovitz M, White C, Barnett RN, Stevens S, Russel E, Vargo D, Flah MH Diet, faecal bile acids, and neutral sterols in carcinoma of the colon Dig Dis Sci 24 746-751, 1979
16 Mudd DG, McKelvey STD, Norwood W, Elmore DT, Roy AD Faecal bile acid concentrations of patients with carcinoma or increased risk of carcinoma in the large bowel Gut 21 587-590, 1980
17 Murray WR, Blackwood A, Trotter JM, Calman KC, MacKay C Faecal bile acids and clostridia in the aetiology of colorectal cancer Br J Cancer 41 923-928, 1980
18 Kaibara N, Sasaki T, Ikeguchi M, Koga S, Ikawa S Fecal bile acids and neutral sterols in Japanese with large bowel carcinoma Oncology 40 255-258, 1983
19 Tanida N, Hikasa Y, Shimoyama T, Setchell KDR Comparison of faecal bile acid profiles between patients with adenomatous polyps of the large bowel and healthy subjects in Japan Gut 25 824 832, 1984
20 Hikasa Y, Tanida N, Ohno T, Shimoyama T Faecal bile acid profiles in patients with large bowel cancer in Japan Gut 25 833 838, 1984
21 Breuer NF, Domes P, Jaekel S, Goebell H Fecal bile acid excretion pattern in colonic cancer patients Dig Dis Sci 30 852-859, 1985
22 Hinton JM, Lennard Jones JI , Young AC A new method for stuyding gut transit time using radioopaque markers Gut 10 842 847, 1969
23 Faassen A van, Nagengast F M, Hectors M, Broek WJH van den, Huybregts AWM, Werf SDJ van der, Berge Henegouwen GP van, Tongeren JHM van Determination of individual human faecal bile acids by gas-liquid chromatography after enzymatic deconjugation and simultaneous solvolysis and methylation using dimethoxypropane Clin Chim Acta 152 231-239, 1985
24 Davignon J, Simmonds WJ, Ahrens EHJr Usefulness of chromic oxide as an internal standard for balance studies in formula fed-patients and for assessment of colonic function J Clin Invest 52 127-138, 1968
25 Hill MJ, Aries VC Faecal steroid composition and its relationship to cancer of the large bowel J Path 104 129-39, 1971
26 Reddy BS, Wynder EL Large-bowel carcinogenesis fecal constituents of populations with diverse incidence rates of colon cancer J Natl Cancer Inst 50 1437-1442, 1973
27 IARC Intestinal Microecology Group Dietary fibre, transit time, faecal bacteria, steroids and colon cancer in two Scandinavian populations Lancet 2 207-211, 1977
28 Reddy BS, Hedges A, Laakso K, Wynder FL Metabolic epidemiology of large bowel cancer Fecal bulk and constituents of high risk North American and low-risk Finnish population Cancer 42 2832 2838, 1978
29 Jensen OM, Maclennon R, Wahrendorf J Diet, bowel function, fecal characteristics and large bowel cancer in Denmark and Finland Nutr Cancer 4 5-19, 1982
30 Domellof L, Darby L, Hanson D, Mathews I , Simi B, Reddy BS Fecal sterols and bacterial β-glucuronidase activity a preliminary metabolic epidemiology study of healthy volunteers from Umea, Sweden and metropolitan New York Nutr Cancer 4 120-127, 1982
31 Werf SDJ van der, Huybregts AHM, Lamers HI M, Berge-Henegouwen GP van, Tongeren JHM van Age dependent differences in human bile acid metabolism and 7α-dehydroxylation Eur J Clin Invest 11 425-431, 1981
32 Dokkum W, Pikaar NA, Thisen JTNM Physiological effects of fibre-rich types of bread 2 Dietary fibre from bread digestibility by the intestinal microflora and water holding capacity in the colon of human subjects Br J Nutr 50 61-74, 1983

CHAPTER IV

Inhibition of secondary bile acid formation in the large intestine by lactulose in healthy subjects of two different age groups

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CHAPTER IV

Inhibition of secondary bile acid formation in the large intestine by lactulose in healthy subjects of two different age groups

Abstract

Secondary bile acids have been implicated in colonic carcinogenesis. Transformation of primary into secondary bile acids (7α-dehydroxylation) in the large bowel is a pH-dependent process. Inhibition of this reaction could be achieved by lowering colonic pH. We, therefore, studied the effects of lactulose (a non-absorbable disaccharide), which is capable of acidifying colonic contents, on secondary bile acid metabolism. Because this metabolism is age dependent, lactulose was given (0,3 $g.kg^{-1}$ twice daily for 12 weeks) to nine middle-aged (age 31-54 years; mean 45.7) and ten elderly subjects (age 56-81 years; mean 66.4). Twice before and after 6 and 12 weeks lactulose administration, biliary and faecal bile acids, whole gut transit time, faecal weight and dry weight, and faecal pH were recorded. The concentration of (iso)lithocholic and deoxycholic acid in faeces was higher in elderly subjects ($p<0.05$), but the excretion was comparable. After lactulose the concentration and excretion of the major secondary bile acids decreased. The primary bile acid fraction rose from 5% before to more than 20% after lactulose ($p<0.05$). Faecal weight increased and faecal dry weight decreased, resulting in a higher faecal water output during lactulose. Whole gut transit time did not change. The faecal pH dropped after 6 ($p<0.05$) and further after 12 weeks lactulose ($p<0.05$). The percentage deoxycholic acid in bile was higher and cholic acid lower in elderly subjects ($p<0.05$). After lactulose the deoxycholic acid content decreased ($p<0.05$) and chenodeoxycholic acid content increased ($p<0.01$) in the whole group ($n=19$). The cholic acid content did not change.
The results show that lactulose can reduce secondary bile acid formation in the large bowel and that the effect is not mediated through acceleration of intestinal transit. Acidification of colonic contents seems to be the major factor in this reduction.

Introduction

In the large intestine the primary bile acids cholic acid and chenodeoxycholic acid

are converted into the secondary bile acids deoxycholic and lithocholic acid by 7α-dehydroxylation [1]. These last compounds have been implicated in colorectal carcinogenesis [2]. The enzyme 7α-dehydroxylase has a pH optimum of 7-8, and is produced by strictly anaerobic bacteria [3, 4]. It has been suggested that a high colonic pH (>7) could promote colorectal carcinogenesis, possibly by enhanced formation of (co)carcinogenic compounds, such as the secondary bile acids [5]. Lowering colonic pH could theoretically inhibit 7α-dehydroxylation and thus reduce the formation of secondary bile acids. Epidemiological studies have demonstrated, that populations at low risk for developing colonic cancer did have a lower faecal pH than those at higher risk [6, 7]. From one of these studies from South-Africa [7], it emerged, that the dietary fibre content of the diet may not be the major determinant of the lower faecal pH in the former group. However, consumption of a diet rich in dietary fibre can reduce faecal pH [8, 9]. The same holds true for lactulose [10]. Lactulose has also been shown to decrease the percentage of biliary deoxycholate in obese females [11]. It has laxative properties as well and the effect on bile acid metabolism could be mediated by this effect [12]. To gain more insight in the mechanisms, by which lactulose influences secondary bile acid metabolism, we studied the effect of lactulose on biliary and faecal bile acid composition, on gut transit time and on faecal pH in healthy volunteers. Because secondary bile acid metabolism is related to age [13, 14], we examined subjects in two different age groups, namely middle-aged and elderly persons.

Subjects and methods

Subjects

Nineteen healthy volunteers gave written consent for participation in the study, which was approved by the hospital Human Research Review Committee. Ten females and nine males of two age groups were studied. One group existed of middle-aged subjects (4 male, 5 female) with a mean age of 45.7 years (range 31-54 years) and a second group of elderly subjects (5 male, 5 female) with a mean age of 66.4 years (range 56-81 years). The two age groups were of comparable body weight (mean 73.2 vs. 74.7 kg) and body mass index (mean 24.7 vs. 26.0 kgm^{-2}). No subject had gastrointestinal complaints and none had undergone gastrointestinal or biliary surgery. A prerequisite for inclusion in the study were normal liver function tests, blood glucose and cholesterol-triglyceride serum levels. No laxatives or antibiotics had been used at least 6 weeks before entry into the study. Body weight was recorded three times during the study and proved to be stable. All subjects consumed their regular diet during the study period.

Design of the study

After 2 weeks (baseline period), in which no lactulose was given and the subjects adhered to their regular diet, each subject received 0.3 g lactulose (Legendal®) per kg body weight, twice daily (total dose 24-48 g day^{-1}) during 12 weeks. In none of the subjects did diarrhoea occur, although in most of them the consistency of the stool became somewhat softer and the defaecation frequency increased from an average of 1.4 to 1.7 times a day (not significant). Stools were collected for the measurement of the whole gut transit time, faecal pH and faecal bile acid analysis. After an overnight fast, bile was collected one day after the stool collection was completed.

All parameters were studied twice before, and after 6 and 12 weeks' lactulose administration. The reason for collecting data twice in the baseline period was to check the physiological variation with time.

Stool collection

Stools were collected at home during 3 days in each weekly period (table I) and immediately after evacuation stored in a box containing dry ice (frozen CO_2; −70 °C). On arrival at the laboratory the samples were radiographed and the radio-opaque pellets counted in order to calculate the whole gut transit time and then stored at −20 °until analysis.

Whole gut transit time

Transit time was measured by the single stool method with radio-opaque pellets according to Cummings and Wiggins [15].

Faecal pH

The 3-day stool samples were pooled, weighted and homogenised with distilled water. A sample was taken and the pH measured with a pH electrode (Radiometer, Copenhagen, Denmark).

Faecal bile acid analysis

After homogenization of the faeces a sample was freeze dried and, following extraction and derivatization, bile acids were analysed by gas-liquidchromatography as published previously [16]. In this assay lithocholic (LCA) and isolithocholic acid (iLCA) can not be separated and are referred to as (iso)LCA.

Biliary bile acid composition

A fasting duodenal bile sample was obtained (2 ml) after gall-bladder stimulation by cholecystokinin (Kabi-Vitrum, Sweden) in a dose of 0.5 IDU kg body $weight^{-1}$ [17]. Bile samples were stored at −20 °C until analysis. Biliary bile acids were measured as described previously by gas-liquid chromatography [17].

Statistics

Differences between the data before and during lactulose administration were evaluated by the paired Wilcoxon Rank sum test; differences between the age; groups by the non-paired Wilcoxon Rank sum test.

Table 1 Major parameters before lactulose administration on 2 consecutive weeks in all subjects (n=19).

	week - 2	week - 1
Faecal bile acids (μmol g^{-1} dry weight)		
(i)LCA	7.54±1.03*	6.68±0.69
DCA	8.54±0.83	8.21±0.34
Biliary bile acids (mol %)		
LCA	3.1±0.6	3.1±0.6
DCA	21.9±2.5	21.2±2.4
CDCA	34.7±1.7	34.5±1.1
CA	40.6±2.7	41.6±2.1
Stool weight (g.day ^{1})	167±16	170±13
Whole gut transit time (hrs)	45±6	38±3
Faecal pH	7.01±0.07	6.99±0.10

Data are expressed as means ± SEM. None of the data are significantly different.
LCA: lithocholic acid, DCA: deoxycholic acid, CDCA: chenodeoxycholic acid, CA: cholic acid, (i)LCA: lithocholic + isolithocholic acid.

Results

No significant differences were found between the major parameters in the two consecutive weeks before lactulose was administered (table 1). Therefore, the results during lactulose administration were compared to the average of the data obtained in the weeks preceding the lactulose period.

Stool analysis, whole gut transit time and stool pH

In nearly all subjects the faecal weight increased after lactulose administration and the faecal dry weight decreased, resulting in a higher faecal water output during lactulose (table 2). No differences were found between elderly and middle-aged subjects in this respect.

Table 2 Stool parameters and intestinal transit time before and during lactulose in middle-aged (MS) and elderly (ES) subjects.

		Before lactulose	During lactulose 6 weeks	During lactulose 12 weeks
Stool weight (g day^{-1})	MS	180±23†	217±31	241±41*
	ES	162±16	199±18	225±26
Stool dry weight (%)	MS	23.8±1.7	20.8±1.5	20.7±1.4*
	ES	22.0±1.2	18.9±1.3*	18.7±1.0*
Stool water (g day^{-1}) Output	MS	140±19	174±28	195±35*
	ES	117±10	171±18*	187±23*
Whole gut transit time MS (hrs)	MS	45.3±7.3	41.1±3.7	48.1±6.4
	ES	37.8±4.2	38.9±4.8	35.9±2.4

* $P<0.05$ vs. before lactulose. Values between weeks 6 and 12 were not significantly different. Parameters between MS and ES were not significantly different before and during lactulose.

† Data are expressed as means ± SEM.

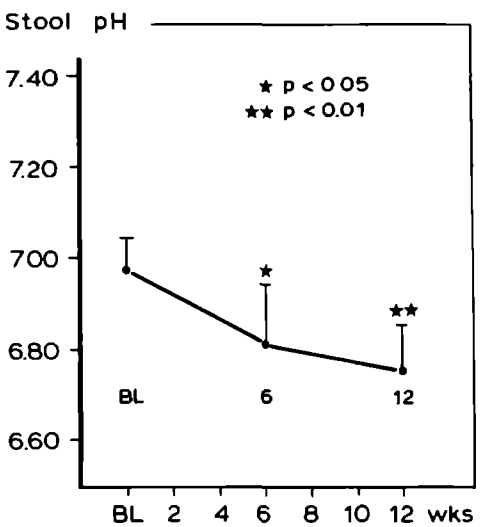

Figure 1 Stool pH before (BL) and after 6 and 12 weeks' lactulose administration in 19 subjects.

The whole gut transit time did not change during lactulose administration in both age groups (table 2) and none of the subjects developed diarrhoea. No significant differences in transit time between the middle-aged and elderly subjects were found before and during lactulose administration. As is shown in figure 1 the stool pH dropped significantly after 6 and even further after 12 weeks lactulose, and this effect was observed in both age groups.

Faecal bile acid analysis

The concentration of the major secondary bile acids in elderly subjects was significantly higher, due to the lower weight of dry faecal material in the elderly persons (table 3). However, no significant differences were present in the excretion of bile acids before lactulose administration between middle- aged and elderly subjects.

In both middle and elderly aged subjects the concentration and excretion of the major secondary bile acids decreased during lactulose, while the primary bile

Table 3 Faecal bile acid pattern before and during lactulose administration in middle-aged (MS) and elderly subjects (ES)

			During lactulose			
	Before lactulose		6 weeks		12 weeks	
Bile acid	MS	ES	MS	ES	MS	ES
Concentration (μmol g^{-1} dry weight)						
(i)LCA	5 39±0 60†	8 52±1 30	3 56±0 66	5 71±1 05	3 31±0 50	5.42±1 35
iDCA	0.93±0 21	1 74±0 30	0 79±0 28	1 46±0 78	0 80±0 29	0 95±0 23
DCA	6.93±0 53	9 76±0 87	4 88±0 78	7 94±0 99	4 63±0 67	6.83±1 09
CDCA	0 25±0 06	0 21±0 09	0 74±0 34	0 41±0 10	0 60±0 25	0 76±0.34
CA	0 57±0 30	0 51±0 34	1.69±0 81	0 50±0 06	1 71±0 68	1 52±0 95
Excretion (μmol day^{-1})						
(i)LCA	214±32	277±37	151±35	221±50	142±26	187±31
iDCA	38±10	56± 9	36±13	50±14	43±20	33± 7
DCA	274±31	323±42	203±40	309±59	197±31	251±34
CDCA	11± 3	7± 3	34±17	14± 3	32±17	33±16
CA	27±15	18±12	75±39	16± 8	87±45	70±44
PBA (%)	6 7	3 6	21 8	4 9	23 7	17 9

P <0 05 MS vs ES; P <0 05 before lactulose.

† Data are expressed as means ±SFM.

(i)LCA. lithocholic + isolithcholic acid, iDCA· isodoxycholic acid, DCA: deoxycholic acid, CDCA· chenodeoxycholic acid, CA cholic acid, PBA(%). fraction primary bile acids

acids tended to rise and after 12 weeks lactulose the primary bile acids came to represent 23.7% ($p<0.05$) and 17.9% ($p<0.05$) of the total bile acid excretion in middle-aged and elderly subjects respectively (table 3). The values did not decrease further after 12 weeks, compared to 6 weeks. No significant differences in concentration and excretion between the elderly and middle-aged subjects were observed during lactulose administration.

Biliary bile acid composition

The biliary bile acid composition before lactulose administration is shown in table 4. Elderly persons had significantly more deoxycholic acid (DCA) and less cholic acid (CA) in their bile. During lactulose administration the DCA content decreased significantly in the total group of 19 subjects after 6 and 12 weeks. In contrast, the chenodeoxycholic acid (CDCA) content increased significantly after 6 and 12 weeks. The molar percentage of CA did not change (fig. 2).

When the two age groups were analysed separately, it was shown that in elderly subjects the DCA content decreased significantly only after 6 weeks (mean 22.2% vs 26.0%; $p<0.05$) since despite the lower content after 12 weeks (mean 18.1% vs 26.0%; $p=0.10$), the decrease of the biliary DCA content was observed

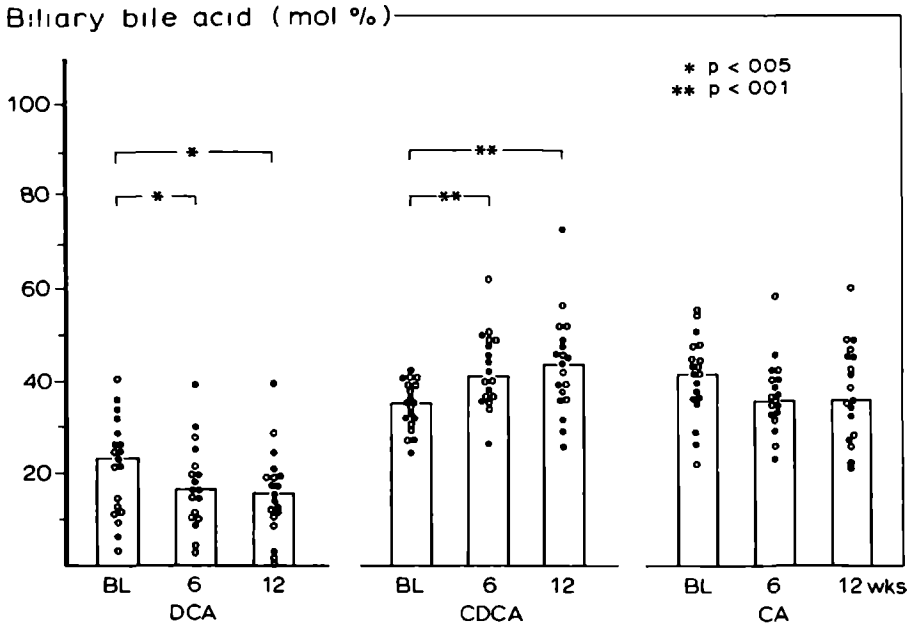

Figure 2 Biliary bile acid composition in middle aged (0) and elderly subjects (●), before lactulose (BL) and during lactulose administration (weeks 6 and 12). For abbreviations see table 4.

Table 4 Baseline biliary bile acid composition (average of week - 2 and - 1, in mol%) in middle-aged (n=9) and elderly (n=10) subjects.

	Middle-aged subjects	Elderly subjects
LCA	2.3±0.2	3.7±0.8
DCA	17.1±3.7	26.0±2.6*
CDCA	35.5±1.7	33.7±3.8
CA	44.7±3.2	37.3±2.2*

* $P < 0.05$.

† Data expressed as means ± SEM.
LCA: lithocholic acid, DCA: deoxycholic acid, CDCA: chenodeoxycholic acid, CA: cholic acid.

only in eight of the ten subjects. In contrast, the CDCA content increased after 6 (mean 41.3% vs 33.7%; $p<0.01$) and 12 weeks (mean 42.7% vs 33.7%; $p<0.05$). In the middle-aged subjects the DCA content did not change after 6 weeks (mean 13.7% vs 17.1%; $p=0.14$) and after 12 weeks (mean 13.4% vs 17.1%; $p=0.08$). In this group in eight of ten subjects the DCA content in bile decreased after 12 weeks. The CDCA content in this group increased after 6 weeks (mean 44.1% vs 35.5%; $p=0.07$) but more so after 12 weeks (mean 44.7% vs 35.5%; $p<0.05$). In both groups the CA content of bile did not change.

Discussion

This study shows that the formation of secondary bile acids in the large intestine can be reduced by the non-absorbable disaccharide lactulose. The evidence for this conclusion is based both on the decreased concentration and excretion of the major secondary bile acids in the stool, and on the decreased proportion of deoxycholic acid in bile. It confirms and extends the previous study of Thornton and Heaton in obese females [11]. The biliary bile acid composition before lactulose administration is in accordance with recent reported data in the literature [18]. The observed difference in DCA content in bile between middle- aged and elderly subjects could be explained by the age dependence of the CA and DCA metabolism, as described previously [13, 18]. Whether the higher DCA and lower CA content in bile in elderly subjects is the consequence of a decreased CA pool size with age [18] or a higher fractional turnover rate of CA and greater DCA input rate with age [13] can not be established from these data, since no kinetic experiments were carried out.

The mechanism by which the reduction of secondary bile acid formation during

lactulose takes place is not completely understood, but the results from this study clearly show that the intestinal transit time (mouth to anus transit) is not reduced during lactulose administration and therefore does not play an important role. It can not be ruled out, however, that lactulose exerts an effect on small bowel transit, as was shown previously [19, 20]. The acceleration of the small intestinal transit time is probably caused by osmotic inhibition of fluid absorption. It has been shown that administration of mannitol (also a poorly absorbed sugar) can decrease small bowel transit time and increase the rate of bile salt excretion [21]. However, the dose (120 g mannitol) used in that study caused diarrhoea and decreased the intestinal transit time (mouth to anus) from 25 to 5 hours. Thus, these results are not comparable to our study, in which no diarrhoea occurred in any of the subjects, probably due to the much lower dose of lactulose. Furthermore, in the study of Read et al [20] no change in whole gut transit and only a moderate decrease of small bowel transit was found during the administration of 40-g lactulose.

A reasonable explanation for the reduction of the secondary bile acid formation in the large bowel is the inhibition of the enzyme 7α-dehydroxylase. This enzyme is responsible for the conversion of the primary into the secondary bile acids. The pH optimum is between 7 and 8. Lactulose is fermented and converted into short chain fatty acids and lactate in the proximal part of the large bowel and can reduce intracolonic pH [10, 22]. Since the acidification of colonic contents by lactulose particularly takes place in the proximal part of the large bowel [10], and absorption of short chain fatty acids and secretion of bicarbonate occurs towards the distal part, the real pH change in the proximal part might even have been greater than is suggested from the stool pH in this study. This could have resulted in an inhibition of the 7α-dehydroxylation and thus in an decreased formation of secondary bile acids. The effect is not always accomplished since in some of our individuals the percentage of deoxycholic acid in bile did not fall. It is possible that in different subjects lactulose is metabolized in different ways [22].

Concomitant with the decrease in biliary DCA, the content of CDCA increased. This finding could be explained by several mechanisms. Originally it was hypothesized that DCA selectively inhibits CDCA synthesis and that reduced formation or absorption of DCA will inhibit the negative feedback on the CDCA synthesis [23]. Others have questioned this hypothesis and it has also been suggested that competition for intestinal transport explains the reciprocal relation between DCA and CDCA in bile [24]. From our data no conclusions can be drawn, since no bile acid kinetic studies were carried out. In the past we have only been able to show an association between a high DCA absorption and low proportion of CDCA in bile in elderly subjects [25].

Whether the effect of lactulose on secondary bile acid metabolism can be reproduced by more natural dietary constituents like fibre remains to be established.

Recently it was shown that different fibre components have different effects on bile salt metabolism [26]. Pectin possibly enhances the formation of secondary bile acids, while cellulose lowers it. Lignin seems to have no effect. Dietary fibre in the form of wheat bran or concentrated wheat fibre has been shown to cause variable changes in secondary bile acid metabolism [27, 28]. The ultimate goal in this respect should be an inhibition of colonic carcinogenesis by lowering colonic secondary bile acid formation. A large prospective dietary intervention trial in subjects at high risk for developing colonic cancer, e.g. patients with adenomatous polyps, might give more insight in this problem.

In conclusion, this study shows that lactulose inhibits secondary bile acid formation in the large intestine. The effect is probably mediated through acidification of colonic contents, thereby reducing 7α-dehydroxylation of primary bile acids.

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References

1. MacDonald IA, Bokkenheuser VD, Winter J, McLernon AM, Mosbach EH. Degradation of steroids in the human gut. J Lipid Res 1983; 24: 675-700.
2. Breuer N, Goebell H. The role of bile acids in colonic carcinogenesis. Klin Wochenschr 1985; 63: 97-105
3. MacDonald IA, Singh G, Mahony DE, Meier CE. Effect of pH on bile salt degradation by mixed fecal cultures. Steroids 1978; 22: 245-56.
4. Midvedt T, Norman A. Parameters in 7t-dehydroxylation of bile acids by anaerobic lactobacilli. Acta Pathol Microbiol Scand 1968; 72: 313-29.
5. Thornton JR. High colonic pH promotes colorectal cancer. Lancet 1981; 1: 1081-3.
6. Walker ARP, Walker BF, Segal I. Faecal pH value and its modification by dietary means in South African black and white school children. S Afr Med J 1979; 55: 495-8.
7. Walker ARP, Walker BF, Walker AJ Faecal pH, dietary fibre intake, and proneness to colon cancer in four South African populations. Br J Cancer 1986; 53: 489-95.
8. Phillips RL,. Role of life-style and dietary habits in risk of cancer among Seventh Day Adventists. Cancer Res 1975; 35: 3513-22.
9. Dokkum van W, Boer de BCJ, Faassen van A, Pikaar NA, Hermus RJJ. Diet, faecal pH and colorectal cancer. Br J Cancer 1983; 48: 109-10.
10. Bown RL, Gibson JA, Sladen GE, Hicks B, Dawson AM. Effects of lactulose and other laxatives on ileal and colonic pH as measured by a radiotelemetry device. Gut 1974; 5: 999-1004.
11. Thornton JR, Heaton KW Do colonic bacteria contribute to cholesterol gallstone formation? Effects of lactulose on bile Br Med J 1981; 1: 1018-20.
12. Marcus SN, Heaton KW. Intestinal transit, deoxycholic acid and the cholesterol saturation of bile-three inter-related factors. Gut 1986; 27: 550-8.

13. Werf van der SDJ, Huybregts AMM, Lamers HLM, Berge Henegouwen van GP, Tongeren van JHM Age dependent differences in human bile acid metabolism and 7t-dehydroxylation. Eur J Clin Invest 1981, 11: 425-31
14. Nagengast FM, Hectors MPC, Werf van der SDJ, Tongeren van JHM Age dependent differences in secondary bile acid concentration in human feces Gastroenterology 1985; 88: 1514.
15 Cummings JH, Wiggins HS Transit trough the gut measured by analysis of a single stool Gut 1976; 17 219-7.
16 Faassen A van, Nagengast FM, Hectors M et al. Determination of individual human faecal bile acids by gas-liquid chromatography after enzymatic deconjugation and simultaneous solvolysis and methylation using dimethoxypropane Clin Chim Acta 1985; 152: 231-9.
17 Huijbregts AWM, Schaik van A, Berge Henegouwen van GP, Werf van der SDJ Serum lipids, biliary lipid composition and bile acid metabolism in vegetarians as compared to normal controls. Eur J Clin Invest 1980; 10 443-50
18 Einarsson K, Nilsell K, Leyd B, Angelin B Influence of age on secretion of cholesterol and synthesis of bile acids by the liver. N Fngl J Med 1985, 313. 277-82
19 Holgate AM, Read NW. Relationship between small bowel transit time and absorption of a solid meal. Influence of metoclopramide, magnesium sulfate and lactulose Dig Dis Sci 1983; 28: 812-9
20 Read NW, Miles CA, Fisher D et al Transit of a meal through the stomach, small intestine and colon in normal subjects and its role in the pathogenesis of diarrhea. Gastroenterology 1980, 79 1276-82.
21 Meihoff WE, Kern F. Bile salt malabsorption in regional ileitis, ileal resection, and mannitol induced diarrhoea J Clin Invest 1968, 47: 261-7.
22. Florent C, Flourie B, Leblond A, Rautureau M, Bernier JJ, Rambaud JC Influence of chronic lactulose ingestion on the colonic metabolism of lactulose in man (an in vivo study) J Clin Invest 1985, 75: 608-13.
23. Pomare EW, Low-Beer TS Selective suppression of chenodeoxycholate synthesis by cholate metabolites in man Clin Sci Mol Med 1975, 48· 315-22
24 Hoffman AF, Lachin JM Biliary bile acid composition and cholesterol saturation. Gastroenterology 1983, 84. 1075-7
25. Werf van der SDJ, Berge Henegouwen van GP, Tongeren van JHM. Colonic absorption of endogenous deoxycholate and biliary lipid composition in man In: Paumgartner G, Stiehl A, Gerok W eds. Bile-acids and cholesterol in health and disease. Lancaster: MTP Press Limited, 1982: 275-7.
26. Hillman LC, Peters SG, Fisher CA, Pomare EW Effects of the fibre components pectin, cellulose and lignin on bile salt metabolism and biliary lipid composition in man. Gut 1986; 27 29-36
27 Marcus SN, Heaton KW. Effects of a new, concentrated wheat fibre preparation on intestinal transit, deoxycholic acid metabolism and the composition of bile. Gut 1986, 27. 893-900
28. Story JA, Thomas JN Modification of bile acid spectrum by dietary fiber In: Vahouny GV and Kritchesvky D eds Dietary Fibre in Health and Disease New York. Plenum Press, 1982: 193-202

CHAPTER V

The effect of a natural high fiber diet on fecal an biliary bile acids, fecal pH and whole gut transit time in man. A controlled study.

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Submitted for publication.

CHAPTER V

The effect of a natural high fiber diet on fecal an biliary bile acids, fecal pH and whole gut transit time in man. A controlled study.

Abstract

Colonic cancer is associated with a high fat and low fiber consumption. The mechanisms by which these dietary factors exert their action are not well known, but an effect through secondary bile acids has been implicated.

We therefore investigated the effect of a natural high fiber diet on secondary bile acid formation in twelve healthy subjects on a habitual low fiber diet (experimental group). Fat consumption was kept stable. A control group of ten persons consumed their habitual high fiber diet. Parameters studied were fecal and biliary bile acid composition, fecal wet and dry weight, fecal pH and whole gut transit time.

Changes in the parameters after 6 and 10 weeks of high fiber consumption versus the initial values in the experimental group were compared to changes in the control group during the same period.

The experimental group showed a significantly greater reduction in the concentration of the major secondary bile acids after 6 and 10 weeks than the control group. The fecal bile acid excretion, however, did not change. The secondary bile acid fraction was not altered. The change in the whole gut transit time in the experimental group after 6 and 10 weeks did not differ from that in the control group. In contrast, the fecal pH showed a significantly greater decline in the experimental than in the control group. Biliary deoxycholic acid (DCA) was reduced and biliary cholic acid (CA) increased after 6 weeks in the experimental group, compared to the change in the control group; however, after 10 weeks biliary DCA increased and biliary CA returned to the baseline value.

This investigation shows that in a controlled study the fecal secondary bile acid concentration can be reduced on a natural high fiber diet. This effect is mediated through an increase in stool wet and dry weight. The 7α-dehydroxylation of primary bile acids is probably not or only transiently inhibited.

Colonic cancer is associated with a high dietary fat and low fiber intake (1-5). Epidemiologic studies cannot clarify whether this association is causal and, if so, by which mechanisms these dietary factors exert their action. A high fat consumption can increase bile acid excretion (6), although results have been controversial (7). Bacterially degraded bile acids in the large intestine like deoxycholic acid (DCA) and lithocholic acid (LCA) can act as tumor promoters in chemically induced carcinogenesis in rats (8-10) and can influence colonic mucosal proliferation (11). A high dietary fiber intake might conceivably protect against colonic carcinogenesis by accelerating intestinal transit, diluting colonic contents and reducing colonic pH (5, 12-15).

The influence of dietary fiber on bile acid metabolism has been studied in several ways. In most studies either biliary bile acid composition or fecal bile acids were measured. Supplementation with bran generally reduced biliary DCA (16-24), although in two studies no effect was observed (21, 22). When separate components of dietary fiber were studied, different effects were observed. Pectin had no effect on biliary bile acids in one (25), but reduced biliary DCA by 40% in another study (26). Cellulose reduced the biliary DCA content and lignin had no effect (26). A natural high fiber diet caused a modest decrease in biliary DCA and an increase in cholic acid (CA) content in gallstone patients (27).

Bran was used in most investigations in which fecal bile acid measurements were performed. In general it caused a decrease in the concentration of bile acids and an unchanged or modestly increased excretion (28-34). The ratio secondary: primary fecal bile acids did not change in studies measuring individual bile acids.

If dietary fiber does play a protective role in colonic carcinogenesis, then the most physiologic way to gain benefit is to increase the natural fiber content of a common average Western diet.

Few data are available on simultaneous measurement of biliary and fecal bile acid profiles in subjects in whom the diet was enriched with fibers from various sources. Moreover, an increase in fiber intake is often accompanied by a decrease in dietary fat consumption, which can influence bile acid metabolism as well (6, 7). Moreover, it is not clear, whether changes in bile acid metabolism occur on a stable nutrient consumption. Therefore a controlled study was performed in which healthy volunteers consuming a low fiber diet increased their fiber intake from various sources during 10 weeks. Simultaneously, a control group consumed their habitual high fiber diet for the same period. Fecal and biliary acids were measured as were whole gut transit time and fecal pH.

Subjects and methods

Design, subjects and statistics

Healthy subjects were recruited through advertisements in local newspapers. A detailed dietary history was taken and individual food consumption patterns were calculated. Subjects were then divided into a control group and an experimental group. The control group consisted of 10 subjects with a habitual fiber intake of more than 3.5 g/MJ (±30 g/day). They followed their habitual diet for the full 14 weeks of the study. The experimental group consisted of 12 subjects with a low habitual fiber intake (less than 3 g/MJ; ±20 g/day). They maintained this habitual low fiber diet for a baseline period of 4 weeks. They were then switched to a high-fiber diet (planned fiber intake 4g/MJ) for the next 10 weeks. Measurements were made in weeks -3 and -1, and again in weeks +6 and +10. For each subject, baseline (initial) values (mean of weeks -3 and -1) were subtracted from values obtained in weeks 6 and 10 to produce individual changes. In the control group values in week -1 were subtracted from values in weeks 6 and 10. The mean change in the experimental group was compared to the mean change in

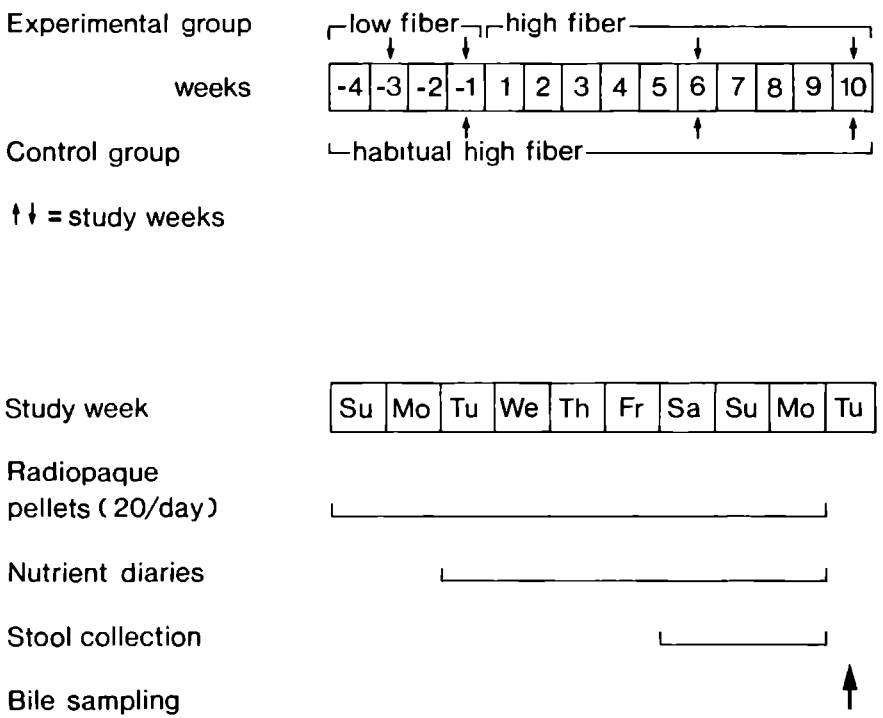

Figure 1 Study-design.

the control group using the Wilcoxon rank sum test (figure 1). Both groups were comparable as to age, sex and relative body wieght (tabel I).
None of the subjects had gastrointestinal complaints or had undergone gastrointestinal or biliary surgery. Physical findings were normal in all. Liver function tests, blood glucose, and cholesterol/triglyceride levels were within the normal range. No laxatives or antibiotics had been used at least 6 weeks before entry into the study.
The study was approved by the hospital Human Research Review Committee and all subjects gave informed written consent.

Table I Baseline data on the subjects studied

	Experimental group	Control group
Number (women/men)	12 (6/6)	10 (5/5)
Age	49.1±4.1	53.9±1.8
Range	33–71	41–62
Weight	71.2±3.3	74.5±3.3
Height	1.72±0.02	1.71±0.03
Body mass index ($kg.m^{-2}$)	24.0±1.0	25.4±0.6

Values represent means ± SEM. No differences between experimental and control group.

Diets
The increase in fiber intake in the experimental group was derived from cereals, fruit and vegetables. Compliance in individual food intake was checked regularly by visiting the subjects at home and discussing possible problems with them. All subjects consumed a common average Dutch diet. During the study period the nature and amount of the daily food intake was noted in a specially designed diary for 7 consecutive days (35). In the control group this was done 3 times (in weeks -1, 6 and 10) and in the experimental group twice during the low fiber period (weeks -3 and -1) and after 6 and 10 weeks on a high fiber diet (see figure 1). Individual food consumption data from the diary were calculated using the National Nutrient Data Base NEVO (36). Care was taken to keep the intake of all nutrients constant with the exception of dietary fiber in the experimental group.

Parameters studied
Parameters studied included stool wet and dry weight, intestinal transit time, fecal pH, total and individual fecal bile acid concentrations and excretion and

biliary bile acid composition. Parameters were studied during the weeks that nutrient diaries were kept at home.

Whole gut transit time and faecal flow correction
On 6 days before and also during stool collection 20 radiopaque pellets were given daily in order to correct for variation in fecal flow (37). On 2 days before stool collection pellets of a different size and shape were given to calculate the whole gut transit time, according to Cummings and Wiggins (38).

Stool collection
Stools were collected at home during 3 days at the end of the 3 (control group) or 4 (experimental group) study weeks. After evacuation feces was immediately frozen in a container with dry ice (frozen CO2; −70 °C). On arrival at the laboratory samples were radiographed and radiopaque pellets counted to calculate the whole gut transit time. The total number of pellets ingested in 3 days (60) was divided by the number of pellets excreted , and the amount of fecal material was multiplied by this value to correct for fecal flow. Samples were then stored at −20 °C until analysis.

Fecal pH
The 3 day stool samples were pooled, weighed and homogenized with distilled water. A sample was taken and the pH measured with a pH electrode (Radiometer, Copenhagen, Denmark).

Fecal bile acid analysis
After homogenization of the feces a sample was freeze-dried and after extraction, bile acids were measured by gas-liquid chromatography (GLC) as described previously (39). A modification was included because the individual bile acids were separated on a capillary column (CP Sil 19 CB, Chrompack, Middelburg, The Netherlands). Bile acids were derivatized and injected as trimethylsilyl ethers. Hyodeoxycholic acid was used as internal standard. An automatic solid injection device (Packard Instruments, Delft, The Netherlands) was used to bring the bile acid derivatives on the column. Helium was used as carrier gas. Injection and flame ionization temperature were 300 °C. A computerized increment of column temperature from 150 ° to 300 °C was used to separate the bile acids. Bile acids were eluted at 275 °C. In this assay all major bile acids, including the iso-bile acids, can be separated completely.

Biliary bile acid analysis
A fasting duodenal bile sample was obtained at the end of each study week after gallbladder contraction had been induced with cholecystokinin in a dose of 0.5

IDU/kg/body weight. Bile samples were stored at −20 °C until analysis and measured on the same capillary column as the fecal bile acids with 7-keto-deoxycholic acid as internal standard. Bile acids were derivatized and injected as trimethylsilyl ethers. Injection and flame ionization temperature were 300 °C. To separate the bile acids an increment of column temperature from 200 °C to 300 °C was used. Bile acids were eluted at 280 °C.

Results

Since no differences were found in the experimental group for all parameters in

Table II Nutrient intake and body weight (means ± SEM) in the experimental and in the control group.

	Experimental group (n=12)			Control group (n=10)		
	Low fiber diet	High fiber diet		Habitual high fiber diet		
	weeks			weeks		
	initial	6	10	initial	6	10
Energy (MJ/day)	8.8±0.7	9.4±0.6	9.1±0.6	9.2±0.5	9.0±0.7	9.1±0.7
Dietary fiber						
g/MJ	2.3±0.2	3.7±0.2	3.8±0.3	3.6±0.2	3.5±0.3	3.5±0.3
g/day	20.3±1.7	34.9±2.9	34.6±2.8	32.3±2.5	30.5±2.3	31.1±2.9
% fibers from vegetabl./fruit	66±3	58±3	57±3	61±3	58±3	55±3
Cholesterol (mg/day)	349±23	291±23	267±23	310±23	249±24	295±38
Fat (energy %)	37±2	37±1	35±2	37±1	37±1	38±2
Carbohydrates (energy %)	43±2	45±2	46±2	45±2	44±2	46±2
Protein (energy %)	15±1	14±1	15±1	15±1	16±1	15±1
Alcohol (energy %)	5±1	5±2	5±1	4±1	4±1	3±1
Body weight (kg)	71.2±11.3	71.2±11.9	72.1±12.3	74.5±10.3	74.4±10.4	75.4±11.0

the two weeks on the low fiber diet (weeks -3 and -1), the values were averaged and results after 6 and 10 weeks of high fiber intake compared to this average value, which will be referred to as initial value.

Dietary assessment

In the control group a stable dietary intake of all food items was achieved, as shown in table II. The dietary fiber intake was fixed at about 3.5 g/MJ. This was the fiber consumption to which the subjects were accustomed. Body weight proved to be stable during the study period.

In the experimental group a slight increase in energy intake during the high fiber period occurred. The dietary fiber intake rose from a mean of 2.3 g/MJ to 3.7 g/MJ after 6 and to 3.8 g/MJ after 10 weeks. In accordance with the experimental design, more than half of the dietary fiber was derived from vegetables and fruit. The intake of all other nutrients proved to be stable. Body weight did not change during the high fiber period (table II).

Table IIIA Fecal wet and dry weight (g/day) (means ± SEM)

	Experimental group (n=12)		Control group (n=10)	
	Fecal wet weight	Fecal dry weight	Fecal wet weight	Fecal dry weight
Initial[1])	106±12	26 9±1 7	174±22	36 9±2 6
Week 6	157±13	37 2±2 6	202±27	42 3±3 7
Change after 6 wks	+ 52±13	+10 2±2 1	+ 27±24	+ 5 3±2 5
Week 10	156±11	36 2±2.1	163±18	36 4±3 0
Change after 10 wks	+ 51±11	+ 9 3±7 4	− 11±14	− 0 5±2 3

Table IIIB Whole gut transit time (hrs) and fecal pH (means ± SEM)

	Whole gut transit time	Fecal pH	Whole gut transit time	Fecal pH
Initial[1])	46 6±3 6	6 92±0.11	44.1±5 7	6 86±0 07
Week 6	44 2±3.3	6 80±0 09	44 8±4 9	6 84±0 16
Change after 6 wks	− 2 4±4 0	−0 12±0 08	+ 0 7±0 8	−0 01±0 13
Week 10	38 9±3 6	6 70±0 10	46 7±5 6	6 88±0 14
Change after 10 wks	− 7 7±3 1	−0 21±0 16	+ 2 6±3 1	+0 02±0 09

1) mean of wk-3/-1 in experimental group and wk-1 in control group significantly different from change in control group ($p<0.05$)

Stool weight, whole gut transit time and fecal pH

Daily stool wet and dry weight, whole gut transit time and fecal pH proved to be stable in the control group throughout the study period as shown in table III A and B.

In the experimental group the decrease in fecal pH was significant after 10 weeks on the fiber enriched diet compared to the changes in the control group. The same applies to the increase in stool weight. The whole gut transit time decreased in the experimental group, but the changes in both groups were not significantly different (table III A and B).

Table IV Individual and total secondary bile acid concentration and daily bile acid excretion (means ± SEM) in healthy volunteers consuming diets of different fiber content.

	Experimental group (n=12)				Control group (n=10)			
Bile acid concentration (μmol/g dry weight)	LCA	isoLCA	DCA	isoDCA	LCA	isoLCA	DCA	isoDCA
Initial[1])	7 08±0 55	3 55±0 34	10 70±1 21	3 51±0 60	4 47±0 30	2 11±0 18	8 80±0 91	2 33±0 74
Week 6	4 62±0 52	2 50±0 25	8 07±1 16	2 06±0 33	5 10±0 29	2 67±0 44	10 12±0 79	2 57±0 61
Change after 6 wks	−2 5 ±0 2	−1 1 ±0 2	2 6 ±0 7	− 1 4 ±0 4	+0 6 ±0 3	+0 5 ±0 4	+1 3 ±0 6	+0 3 ±0 5
Week 10	4 63±0 46	2 43±0 23	8 18±0 94	2 12±0 36	5 14±0 36	2 20+0 22	9 32±0 99	2 34+0 66
Change after 10 wks	−2 5 ±0 3	−1 1 ±0 3	2 5 ±0 6	−1 4 ±0 3	+0 7 ±0 3	+0 1 ±0 1	+0 5 ±0 8	+0 0 ±0 3

	Experimental group (n=12)				Control group (n=10)			
Secondary bile acid concentration and total bile acid excretion (mmol/day)	μmol/g dry weight	μmol/g wet weight	total bile acid excretion	% secondary bile acids	μmol/g dry weight	μmol/g wet weight	total bile acid excretion	% secondary bile acids
Initial[1])	24 8 ±2 1	6 6 ±0 6	0 83±0 08	97 5 +0 6	17 7 ±1 8	4 0 ±0 5	0 72±0 06	97 3 ±1 0
Week 6	17 3 ±1 8	4 2 ±0 5	0 81±0 09	96 9 ±1 4	20 5 ±1 2	4 5 ±0 3	0 93±0 14	95 3 ±2 1
Change after 6 wks	−7 5 ±0 9	−2 4 ±0 4	−0 02±0 08	−0 6 ±1 1	+2 8 ±0 4	+0 5 ±0 1	+0 21±0 11	−2 0 ±1 5
Week 10	17 4 ±0 5	4 1 ±0 4	0 74±0 09	95 5 ±2 0	19 0 ±1 8	4 4 ±0 4	0 91±0 16	95 2 ±1 5
Change after 10 wks	−7 4 ±1 1	−2 5 ±0 5	−0 09±0 07	−2 0 ±1 6	+1 3 ±0 3	+0 4 ±0 1	+0 19±0 14	−2 1 ±1 3

1) mean of wk 3/-1 in experimental group and wk-1 in control group

significantly different from change in control group ($p<0.05$)

(iso)LCA (iso)lithocholic acid, (iso)DCA (iso)deoxycholic acid

Fecal bile acid analysis

The secondary bile acid concentration (both as μmol/g dry weight and μmol/g wet weight) in the control group showed only minor variations during the study (table IV). The total amount of fecal bile acids excreted increased slightly after 6 weeks and stabilized by week 10. In the individual bile acid pattern, no major changes were observed.

In the experimental group the decrease in secondary bile acid concentration was significant by 6 and 10 weeks, compared to the changes in the control group. The total bile acid excretion did not change. The data on individual bile acid concentrations indicate that the decrease after 6 and 10 weeks was significant, compared to changes in the control group. The secondary bile acid fraction did not change in either group (table IV).

Table V Molar percentage of biliary bile acids (means ± SEM) in healthy volunteers consuming diets of different fiber content

	Experimental group (n=12)				Control group (n=10)			
Bile acid	LCA	DCA	CDCA	CA	LCA	DCA	CDCA	CA
Initial[1])	0.3±0.3	27.0±2.4	41.7±1.7	30 9±3.2	1.0±0.3	20.2±3.6	41.6±4.2	37.2±4.3
Week 6	0.2±0.1	22.9±2.9	38 7±1.8	38.1±3.1	0.8±0.3	23.6±3.9	41.4±3.7	34.2±4.6
Change after 6 wks	−0.1±0.1	−4 1±1.8	−3.0±1.2	+7.2±2.1	−0.2±0.2	+3.4±3.1	−0.2±2.5	−2.9±1.7
Week 10	0.2±0.1	24.9±3.4	43.4±2.5	31 5±3.3	0.9±0.3	22.8±4.7	41.5±4.8	34.8±5.4
Change after 10wks	−0.1±0.1	−2.1±2.1	+1.6±2.4	+0.6±2.7	−0.1±0.1	+2.4±2.3	−0.1±3.1	−2.4±2.5

1) mean of wk-3/-1 in experimental group and wk-1 in control group

* significantly different from change in control group ($p<0.05$)

For abbreviations see text.

Biliary bile acid composition

As shown in table V in the experimental group the biliary DCA percentage decreased significantly after 6 weeks, compared to the change in the control group, but not after 10 weeks. The CA content increased significantly after 6 weeks compared to the control group, but at 10 weeks the CA content had returned to the baseline level.

There were no significant differences as to the changes in CDCA content in bile after 6 and 10 weeks between both groups (table V).

Discussion

It has been suggested that the putative protective effect of dietary fiber against colonic carcinogenesis might partly be explained by its effect on bile acid metabolism. However, the reported effects are variable and complex (12). Possible reasons are that different kinds of fiber or components of fiber were tested in various kinds of subjects for various lengths of time. Rarely, control groups in which no modifications of diet were introduced, were analysed simultaneously. Fiber is present in many food substances (like wheat, fruit and vegetables) and consists of many different components (like celluloses, hemi-celluloses, pectin, lignin), which can exert opposite effects on bile acid metabolism (26). In most studies reported, large quantities of bran were used, which cannot be easily incorporated into a normal diet. Moreover, an increased bran intake almost inevitably leads to a lower fat intake (40). This fact makes the interpretation of the data on changes of bile acid metabolism uncertain. Furthermore, compliance for the intake of large amounts of bran in the prevention of colonic diseases is likely to be limited.

Increasing the natural fiber content of the diet seems to be the most physiologic and practical way to influence secondary bile acid metabolism and indirectly (we hope) colonic carcinogenesis. In studies using dietary fiber from various sources (cereals, vegetables and fruit), no effect on total fecal bile acid excretion was found and effects on bile acid concentration were not reported (40, 41). Biliary DCA was modestly reduced on a natural high fiber diet, suggesting a decreased formation and/or absorption of DCA in the large bowel (27). However, in this last study fecal bile acids were not measured.

This controlled study shows that biliary bile acid composition can be changed on a natural high fiber diet. However, the reduction of biliary DCA and increase in CA content of bile appeared to be only transient, since after 10 weeks CA returned to baseline values and the decrease in biliary DCA content diminished compared to the value after 6 weeks. Changes observed at 6 weeks are in agreement with those of a previous study in gallstone patients (27). Marcus and Heaton (23) have shown also that administration of a concentrated wheat fiber preparation reduced the proportion biliary DCA, but that the DCA pool did not change after 6 weeks. The input of DCA from the bowel increased. Since the total bile acid pool increased, as did the cholic acid content of bile, these authors assumed that the cholic acid pool increased. If the same effects had occurred in our study, then the fall in the molar percentage of biliary DCA could have been explained by an expansion of the cholic acid pool after 6 weeks, thereby reducing the proportion of DCA in bile. As we did not carry out kinetic experiments, this explanation remains speculative.

The changes in biliary bile acid composition after 6 weeks were not accompanied

by a reduction of the secondary bile acid excretion which suggests, that secondary bile acid formation was not reduced These findings are at odds with a previous investigation, in which we have demonstrated that lactulose – a non-absorbable disaccharide – can reduce biliary DCA by about 25% and increase the proportion of primary bile acids in the stool to 20% This inhibiting effect on DCA formation was probably mediated by acidification of colonic contents (42) In the present study, despite the fall in fecal pH in the experimental group, colonic pH probably did not change enough to inhibit 7α-dehydroxylation of primary bile acids Dietary fiber is only partly digested by the colonic flora (12)

The decrease in secondary fecal bile acid concentration (expressed both in dry and wet weight) during extra fiber consumption in the experimental group compared to the control group, is in agreement with data previously reported in the literature (24, 28-30, 32, 34) However, in contrast to this study bran was used as the main fiber source in most studies The decrease is caused by the increase in stool output, both in water and dry fecal material The total daily excretion of bile acids did not change which is in accordance with some (28, 43-45), but in contrast to other bran and mixed diet studies, in which a dose-related increase was found in fecal bile acid excretion (29-31, 47).

In conclusion, this study shows that the concentration of the major secondary fecal bile acids can be reduced by adding extra fiber from various sources to the diet The maximum amount of extra fiber that could be incorporated into the diet, without compromising compliance was about 15 g per day The 7α-dehydroxylation of primary bile acids was probably not or only transiently inhibited.

References

1 Wynder EL, Reddy BS Dietary fat and fiber and colon cancer Semin Oncol 1983, 10 264-72

2 Willett WC, MacMahou B Diet and Cancer An overview New Engl J Med 1984, 310 697-703

3 Walker ARP Colon cancer and diet, with special reference to intakes of fat and fiber Am J Clin Nutr 1976, 29 1417-26

4 Jensen OM, MacLennan R, Wahrendorf J Diet, bowel functions, fecal characteristics and large bowel cancer in Denmark and I inland Nutr Cancer 1982, 4 5-19

5 IARC Intestinal Microecology Group Report Dietary fibre, transit time, faecal bacteria, steroids and colon cancer in two Scandinavian populations Lancet 1977, 2 207-11

6 Cummings JH, Wiggings HS, Jenkins DJA, Hill MJ, Pearson JR Influence of diets high and low in animal fat on bowel habits, gastro-intestinal transit, faecal microflora, bile acid and fat excretion J Clin Invest 1978, 61 953 63

7 Brussaard JH, Katan MB, Houtvast JGAJ I aecal excretion of bile acids and neutral steroids on diets differing in type and amount of dietary fat in young healthy persons Eur J Clin Invest 1983, 13 115-22

8 Deschner EE, Cohen BI, Raicht RF Acute and chronic effect of dietary cholic acid on colonic epithelial cell proliferation Digestion 1981, 21 290-6

9 Reddy BS, Watanabe K Effect of cholesterol metabolites and promoting effect of lithocholic acid in coloncarcinogenesis in germfree and conventional F344 rats Cancer Res 1979, 39 1521-4
10 Cohen BR, Raicht RF Effect of bile acids on colon carcinogenesis in rats treated with carcinogens Cancer Res 1981, 41 3759-60
11 Lipkin M Proliferation and differentation of normal and diseased gastrointestinal cells In Physiology of the gastrointestinal tract Ed L Johnson Raven Press New York 1987, 255-84
12 Kelsay JL A review of research on effects of fiber intake in man Am J Clin Nutr 1978, 31 142-59
13 Walker ARP, Walker BF, Segal I Faecal pH value and its modification by dietary means in South African black and white school children S Afr Med J 1979, 55 95 8
14 Walker ARP, Walker BF, Walker AJ Faecal pH, dietary fibre intake, and proneness to colon cancer in four South African populations Br J Cancer 1986, 53 489-95
15 Thornton JR High colonic pH promotes colorectal cancer Lancet 1981, 1 1081-83
16 Pomare FW, Heaton KW Alteration of bile salt metabolism by dietary fibre (bran) Br Med J 1973, 4 262-4
17 Pomare EW, Heaton KW, Low-Beer TS, Espiner HJ The effect of wheat bran upon bile salt metabolism and upon the lipid composition of bile in gallstone patients Dig Dis 1976, 21 521-6
18 McDougal RM, Yakymyshyn L, Walker K, Thurston OG Effect of wheat bran on serum lipoproteins and biliary lipids Can J Surg 1978, 21 433-5
19 Watts JMCK, Jablonski P, Toouli J The effect of added bran to the diet on the saturation of bile in people without gallstones Am J Surg 1978, 135 321-4
20 Wicks ACB, Yeates J, Heaton KW Bran and bile time-course of changes in normal young men given a standard dose Scand J Gastroent 1978, 13 289-92
21 Huybregts AWM, Berge-Henegouwen van GP, Hectors MPC, Schaik van A Effects of a standardized wheat bran preparation in biliary lipid composition and bile acid metabolism in young healthy males Eur J Clin Invest 1980, 10 451 8
22 Arffmann S, Hojgraard L, Siese B, Krag E Effect of oat bran on lithogenic index of bile and bile acid metabolism Digestion 1983, 28 197 200
23 Marcus SN, Heaton KW Effects of a new, concentrated wheat fibre preparation on intestinal transit, deoxycholic acid metabolism and the composition of bile Gut 1986, 27 983-900
24 Tarpila S, Miettinen TA, Metsaranta I Effects of bran on serum cholesterol, faecal mass, fat, bile acids and neutral sterols, and biliary lipids in patients with diverticular disease of the colon Gut 1978, 19 137-45
25 Miettinen TA, Tarpila S Effect of pectin on serum cholesterol, faecal bile acids and biliary lipids in normolipidemic and hyperlipidemic individuals Clin Chim Acta 1977, 79 471 7
26 Hillman LC, Peters SG, Fisher CA, Pomare EW Effects of the fibre components pectin, cellulose, and lignin on bile salt metabolism and biliary lipid composition in man Gut 1986, 27 29-36
27 Thornton JR, Emmet PM, Heaton KW Diet and gall stones effects of refined and unrefined carbohydrate diets on bile cholesterol saturation and bile acid metabolism and upon the lipid composition of bile in gallstone patients Dig Dis 1976, 21 521-6
28 Eastwood MA, Kirkpatrick JR, Mitchell WD, Bone A, Hamilton T Effects of dietary supplements of wheat bran and cellulose on faeces and bowel function Br Med J 1973, 4 392-4
29 Jenkins DJA, Hill MS, Cummings JH Effect of wheat fiber on blood lipids, fecal steroid excretion and serum iron Am J Clin Nutr 1975, 28 1408-11
30 Cummings JH, Hill MJ, Jenkins DJA, Pearson JR, Wiggins HS Changes in fecal composition and colonic function due to cereal fiber Am J Clin Nutr 1976, 29 1468-73

31 Cummings JH, Hill MJ, Jivraj T, Houston H, Branch WJ, Jenkins DJA The effects of meat protein and dietary fiber on colonic function and metabolism I changes in bowel habit, bile acid excretion, and calcium absorption Am J Clin Nutr 1979, 32 2086-93
32 Kretsch MJ, Crawford L, Calloway DH Some aspects of bile acid jand urobilinogen excretion and fecal elimination in men given a rural Guatemalan diet and egg formulas with and without added oat bran Am J Clin Nutr 1979, 32 1492 96
33 Kirby RW, Anderson JW, Sieling B et al Oat bran intake selectively lowers serum low density lipoprotein cholesterol concentrations of hypercholesterolemic men Am J Clin Nutr 1981, 34 824-29
34 Reddy BS, Sharma C, Simi B et al Metabolic epidemiology of colon cancer effect of dietary fiber on fecal mutagens and bile acids in healthy subjects Cancer Res 1987, 47 644-48
35 Staveren van WA, Hautvast JGAJ, Katan MB, Montfort van MAJ, Oosten-van der Goes van HGC Dietary fiber consumption in an adult Dutch population a methodological study using a seven-day record J Am Diet Assoc 1982, 80 324 30
36 NEVO-tabel 1985 The Hague Voorlichtingsbureau voor de Voeding, 1985
37 McPherson Kay R, Jacobs M, Katan MB, Lewis B Relationship between changes in plasma lipoprotein concentrations and fecal steroid excretion in man during consumption of four experimental diets Atherosclerosis 1985, 55 15-23
38 Cummings JH, Wiggins HS Transit through the gut measured by analysis of a single stool Gut 1976, 17 219 23
39 Faassen van A, Nagengast FM, Hectors M et al Determination of individual human faecal bile acids by gas-liquid chromatography after enzymatic deconjucation and simultaneous solvolysis and methylation using dimethoxypropane Clin Chim Acta 1985, 152 231-39
40 Stasse-Wolthuis M, Hauvast JGAJ, Hermus RJJ et al The effect of a natural high-fiber diet on serum lipids, fecal lipids, and colonic function Am J Clin Nutr 1979, 32 1881-88
41 Raymond TL, Connor WI , Warner S, Fry MM, Connor SL The interaction of dietary fibers and cholesterol upon the plasma lipids and lipoproteins, sterol balance, and bowel function in human subjects J Clin Invest 1977, 60 1429 37
42 Nagengast FM, Hectors MPC, Buys WCAM, Tongeren van JHM Inhibition of secondary bile acid function in the large intestine by lactulose in healthy subjects of two different age groups Fur J Clin Invest 1988, 18 56-61
43 Walters RL, McLean-Baird J, Davies PS et al Effects of two types of dietary fibre on faecal steroid and lipid excretion Br Med J 1975, 2 536-8
44 McLean-Baird J, Walters RI , Davies PS, Hill MJ, Drasar BS, Soutgate DAT The effects of two dietary fiber supplements on gastrointestinal transit, stool weight and frequency, and bacterial flora, and fecal bile acids in normal subjects Metabolism 1977, 26 117-28
45 Kay RM, Truswell AS The effect of wheat fibre on plasma lipids and faecal steroid excretion in man Br J Nutr 1977, 37 227-35
46 Jacobson EA, Newmark HL, Bright-See F, McKeown Eyssen G, Bruce WR Biochemical changes as a result of increased fiber consumption Nutr Rep Int 1984, 30 1049-59
47 Spiller GA, Story JA, Wong I G et al Effect of increasing levels of hard wheat fiber on fecal weight, minerals and steroids and gastrointestinal transit time in healthy young women J Nutr 1986, 116 778-85

CHAPTER VI

Relationship between biliary and serum bile acids. Prediction of the molar percentage of deoxycholic acid (DCA) in bile from the DCA content in serum.

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CHAPTER VI

Relationship between biliary and serum bile acids. Prediction of the molar percentage of deoxycholic acid (DCA) in bile from the DCA content in serum.

Abstract

The biliary deoxycholic acid (DCA) level is a reflection of the formation and absorption of this bile acid in the large bowel and could serve as a marker for the exposure of colonic mucosa to secondary bile acid metabolites, which have been incriminated in colonic carcinogenesis.

For the study of biliary bile acid composition, duodenal intubation is a prerequisite. This invasive procedure limits its application in human studies on a larger scale. Serum bile acid analysis would overcome this drawback if a significant and reproducible correlation with biliary bile acid composition would be present. We found a significant and reproducible correlation between biliary and serum DCA in nineteen healthy volunteers (10 f; 9 m, age range 31-81 years) before and during lactulose administration. The equation of the correlation was Y (biliary DCA) = 2.7+0.56 X (serum DCA). Within 8% variation the molar percentage of biliary DCA could thus be predicted from the serum content of DCA. The primary bile acids chenodeoxycholic acid (CDCA) and cholic acid (CA) showed a much weaker correlation. Changes in biliary DCA during lactulose were equalled by changes in serum DCA content.

This study shows that the biliary DCA content can reliably be predicted from serum DCA analysis. Potential applicability could be the investigation of DCA metabolism in patients at high risk of developing colonic cancer and the manipulation of DCA formation by dietary means.

Introduction

High concentrations of secondary bile acids, e.g. deoxycholic acid (DCA), in the large bowel have been implicated in colorectal carcinogenesis. Evidence for this role has been presented from epidemiological, experimental and metabolic investigations (1). DCA originates from cholic acid (CA) by 7 α-dehydroxylation by

the anaerobic flora of the large bowel (2). It is partly absorbed in the colon and enters the enterohepatic circulation (3, 4). The DCA content in bile reflects the amount of this bile acid formed and/or absorbed in the large bowel. The absorption of newly formed unconjugated DCA increases with advancing age (4) and is higher in patients with colonic adenomas, compared to age- and sex-matched controls (5). DCA, which is not absorbed, will be excreted in the faeces. The faecal concentration of DCA and other secondary bile acids increases with age as well, probably because of changes in dietary patterns with advancing age (6). Since the biliary DCA level is a reflection of the amount of DCA formed and absorbed in the large bowel, it could be used as a marker for exposure of the colonic mucosa to potentially harmful secondary bile acid metabolites.

To investigate biliary bile acids, duodenal intubation for bile sampling is a prerequisite. This invasive procedure, however, limits its application in human studies on a larger scale. Serum DCA analysis would overcome this drawback if a significant and reproducible correlation with biliary DCA measurements would be present. It has previously been demonstrated that, during bile acid administration for gallstone dissolution, biliary and serum bile acids were correlated. The relationship was found for DCA, chenodeoxycholic acid (CDCA), CA and ursodeoxycholic acid (UDCA) (7-9).

We studied serum and biliary bile acid composition simultaneously in healthy subjects in a protocol in which the effect of a non absorbable disaccharide, lactulose, on biliary and faecal bile acid profiles was investigated (10).

Material and methods

Collection of fasting bile and serum samples

Nineteen healthy volunteers took part in the study (10 females and 9 males; age range 31-81 yrs). Inclusion criteria for entrance in the study were published previously, as was the study design (10). The study was approved by the hospital Human Research Review Committee and all subjects gave informed written consent. The outline of the study was as follows: on 2 consecutive weeks before and after 6 and 12 weeks of lactulose administration, fasting bile and serum samples were taken for analysis of bile acid profiles. Fasting bile samples were obtained after duodenal intubation and gallbladder contraction with cholecystokinin. Fasting serum samples were obtained simultaneously from an antecubital vein. Bile acid composition before and after lactulose in bile and serum was compared and the correlation between bile and serum for the bile acids DCA, CDCA and CA was calculated.

Serum bile acid analysis

To 0.1 ml (10 μmol/l) 7α, 12α-dihydroxy- 5β-cholanoic acid in methanol (internal standard, Calbiochem., USA) dried under a stream of nitrogen, 1 ml serum was added. After addition of 9 ml 0.5 Mol potassium phosphate buffer (pH 7.0) and heating at 68 °C for 20 minutes, bile acids were extracted by means of SepPak C18 cartridges with 5 ml (70%) methanol, according to Tangerman et al (11). The sample was dried under nitrogen and 1 ml sodium acetate buffer (pH 5.7), containing 0.5 mg/ml purified cholylglycine hydrolase (Sigma, St. Louis, USA) was added to the residu. Deconjugation was performed overnight at 37 °C. After deconjugation the pH was adjusted to 3.5 (with 0.5 N HCL) and the sample was taken through a Lipidex 1000 column (2.5 x 1 cm) according to Setchell (12). After washing the Lipidex columns with 3 x5 ml 0.5 N HCL (pH 3.5) and 3 x 5 ml distilled water, the deconjugated bile acids were extracted with 25 ml 70% methanol. The samples were dried by means of a rotary evaporator. After redissolving the bile acids in 1 ml methanol, they were transferred to small tubes and dried. Hereafter, bile acids were dissolved in methanol (1 ml) and converted to methylesters by adding 2,2-dimethoxypropane (1 ml) and HCL (0.05 ml 37%) and incubating the mixture for 1 hour at room temperature (13). After methylation the samples were dried under nitrogen at room temperature to prevent the formation of pigment polymers. Trimethylsilylation of the methylated bile acids was performed with 0,05 ml of the supernatant of a centrifuged (4500 g; 15 min.) mixture of pyridine-HMDS-TMCS (3:2:1 v/v) at 60 °C for 30 minutes. Immediately before application to the gas chromatograph (GLC) the silylation reagent was dried under nitrogen and the samples redissolved in 50 μl n-hexane. Separation and quantitation of individual bile acids was performed on a Packard 430 GLC (Packard Instruments, Delft, The Netherlands) with flame-ionisation detector and equipped with a 25 m x 0.22 mm i.d. glasscapillary column CP-Sil-5 CB (Chrompack, Middelburg, The Netherlands). This GLC was equipped with an automatic solid injection system (Packard Instruments, Delft, The Netherlands). Helium was used as carrier gas. The injection temperature was 285 °C and a computer based stepwise increment of column temperature was used from 150-290 °C to separate the bile acids. The flame ionisation temperature was 280 °C. The internal standard was eluted before all bile acids at 18 minutes. Elution of bile acids took place between 20 and 26 minutes at 270 °C.

Biliary bile acid analysis

A fasting duodenal bile sample (2 ml) was obtained after gallbladder stimulation by cholecystokinin (Kabi-Vitrum, Sweden) in a dose of 0.5 IDU/kg body weight^{-1}. Bile samples were stored at −20 °C until analysis. Biliary bile acids were measured by gas liquid chromatography as described previously (14).

Statistical Methods

Linear regression analysis of bile acid profiles in serum and bile was carried out with Pearson's correlation coëfficient R. Data before and after lactulose were analysed by the paired Wilcoxon rank sum test.

Results

Serum bile acid analysis

As shown in fig. 1a and 1b, complete separation of the major serum bile acids can be achieved with capillary gas liquid chromatography. The recovery of all added (un)conjugated bile acids was acceptable as shown in table I. To correct for losses, a standard mixture of conjugated and unconjugated bile acids was taken through every procedure. The reproducibility and precision of the assay was investigated by duplicate analysis of a pooled serum sample (total bile acid con-

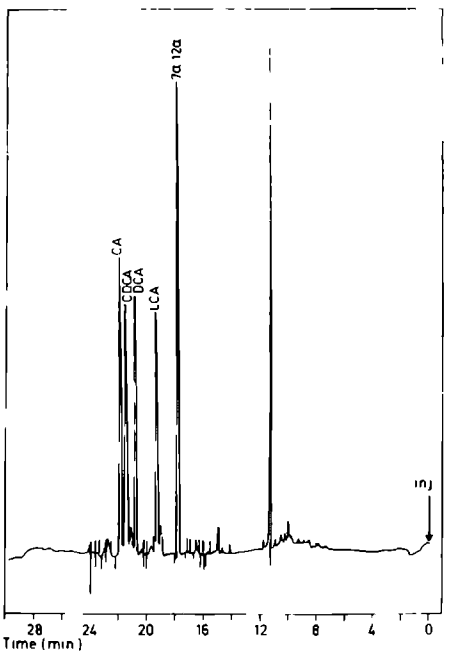

Figure 1a Separation of a mixture of unconjugated bile acids on a gas chromatograph equipped with a capillary column CP-Sil-5 CB. 7α12α: 7α, 12α dihydroxy-5β-cholanoic acid (internal standard); LCA: lithocholic acid; DCA: deoxycholic acid, CDCA: chenodeoxycholic acid; CA: cholic acid.

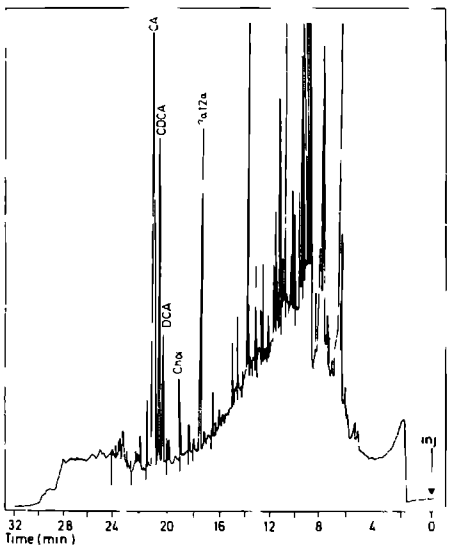

Figure 1b Separation of bile acids extracted from serum. Concentration internal standard (7α12α) 1 μmol/l. Chol: cholesterol.

Table I Recovery of conjugated and unconjugated free bile acids added to serum

Bile acid	Percentage recovered	
	free bile acids	conjugated bile acids
DCA	86 0± 6 5	82 3±3 7
CDCA	85 3±13 2	68.0±2 8
CA	91 0± 3 6	86 3±4.2

Means ± SD of three duplicate measurements To 1 ml serum, 2 5 μmol/l of each bile acid was added
Conjugated bile acids mixture of glyco- and tauro-conjugated of each bile acid (2 1)

centration 4 3 μM) on 8 different days and coefficients of variation of 7.2%, 5 4% and 7.6% were obtained for DCA, CDCA and CA respectively. The amount of other bile acids (like LCA and UDCA) was less than 5% of the total amount and will not be mentioned further.

Correlation between biliary and serum bile acids

As shown in fig 2, a significant and reproducible correlation was found between the molar percentages of DCA in serum and bile both before and during lactulose When all samples were taken together the equation of this correlation was: Y (biliary DCA)=2 7 + 0 56 X (serum DCA), as shown in figure 3 Confidence limits showed that within 8% variation the molar percentage of biliary DCA could be predicted from the serum value using this equation The correlation coefficient for CDCA in serum and bile was less reproducible and CA showed the least significant and reproducible correlation (table II) The serum concentration of DCA decreased significantly after 12 weeks lactulose administration

Table II Correlations between percentage of biliary and serum bile acids before (weeks -2, -1) and during lactulose (weeks +6, +12)

Percentage bile acid	wk-2	wk-1	wk+6	wk+12
%DCA	0.93	0.94**	0.91	0 92*
%CDCA	0 63	0 63	0 92	0 85**
%CA	0 53	0 63	0.67*	0.63*

* p<0 05
** p<0 001

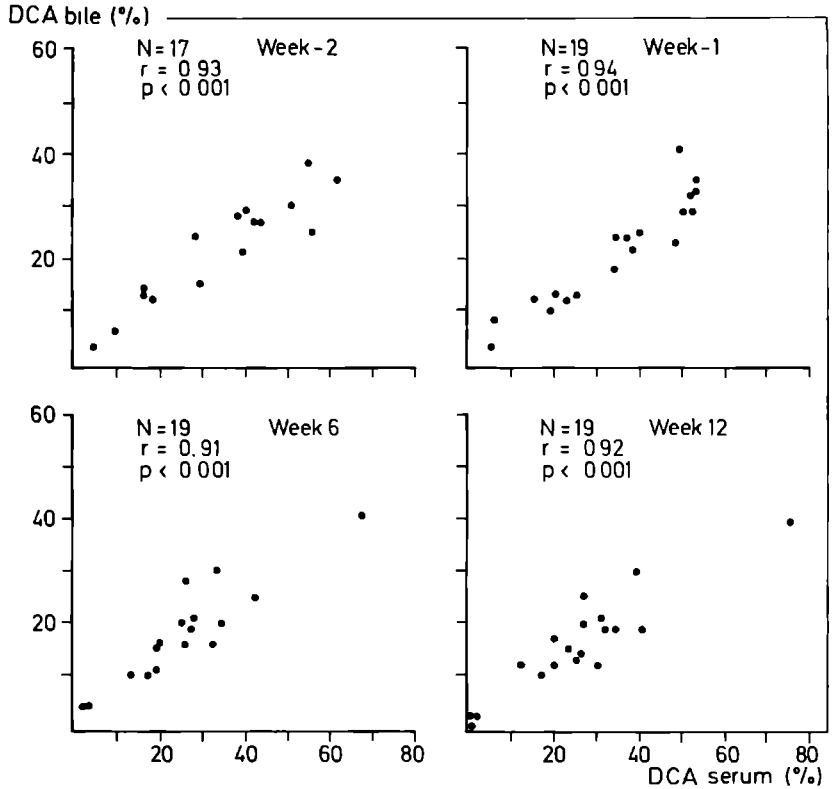

Figure 2 Correlation of molar percentages DCA in serum and bile, before (week -2 and week -1) and during lactulose administration (week 6 and week 12).

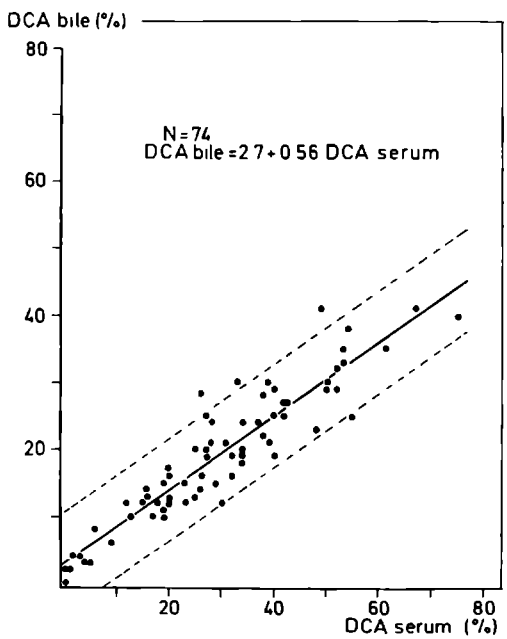

Figure 3 Correlation of molar percentages DCA in serum before and during lactulose with 95% confidence limits.

(table III). No significant changes in the concentration of CDCA and CA were observed. The reduction of the biliary DCA content during lactulose was equalled by the decline of the percentage of DCA in serum after 6 and 12 weeks (table IV). In contrast, the proportion CDCA in serum increased significantly after 6 and 12 weeks lactulose.

Discussion

The serum level of DCA is composed of unconjugated DCA absorbed from the large bowel, where it is formed after deconjugation and 7 α-dehydroxylation of CA and deconjugation of circulating conjugated DCA (3, 4). Conjugated DCA in serum is derived from DCA absorbed in the small bowel from the circulating DCA pool (3). The relative proportions of bile acids in blood and bile are not identical for two main reasons. Firstly, differences exist in the intestinal absorption pattern for individual bile acids (15). A dihydroxy-bile acid bypass of the hepato-ileal circuit is present, which leads to elevated CDCA and DCA levels in serum relative to CA (16). This bypass may be due to passive absorption of

Table III Serum concentration of bile acids (μmol/l; means ± SEM) before (weeks -2, -1) and during lactulose (weeks +6, +12)

Bile acid	wk-2	wk-1	wk+6	wk+12
DCA	0.65±0.09	0.51±0.07	0.46±0.07	0.41±0.06*
CDCA	0.84±0.13	0.84±0.18	0.82±0.10	1.11±0.20
CA	0.65±0.16	0.45±0.10	0.58±0.14	0.50±0.10

* $p<0.05$ vs wks -2/-1.

Table IV Percentage of serum bile acids (means ±SEM) before (weeks -2, -1) and during lactulose (weeks +6, +12).

Bile acid (%)	wk-2	wk-1	wk+6	wk+12
DCA	34.4±3.7	34.3±4.1	26.9±3.7**	25.2±3.9**
CDCA	40.0±2.5	42.7±3.5	46.4±3.7*	52.2±3.1**
CA	25.8±2.6	23.1±2.2	26.7±3.1	22.7±2.1

* $p<0.05$ vs wks -2/-1
** $p<0.01$ vs wks -2/-1

glyco-conjugates of CDCA and DCA in the jejunum (16, 17). Secondly, individual bile acids may be absorbed to different extents from the portal circulation at the liver sinusoidal surface. The fractional hepatic uptake of dihydroxy-bile acids CDCA and DCA is lower than that of CA, and unconjugated bile acids are less efficiently cleared from the portal circulation than conjugated ones (3, 17). Because of these phenomena it is obvious that serum bile acid levels are relatively enriched in conjugated CDCA, DCA and probably also in unconjugated DCA.

In this study it is shown that the molar percentage of DCA in fasting serum and bile is significantly and reproducibly correlated, despite the complex nature of its metabolism (18-19). Moreover, changes in biliary DCA during lactulose administration were equalled by changes in serum DCA. Changes in DCA metabolism that occur by manipulation of external factors (like diet) can thus be observed from serum bile acid measurements. This study confirms and extends a previous study, in which a significant correlation was found between serum and biliary DCA in 31 gallstone patients (7). The correlation between serum and bile for the other major bile acids CDCA and CA were less impressive in this study and differ from the results of Whiting and Watts (7). An explanation could be that these authors studied gallstone patients with a different biliary bile acid pattern than our healthy subjects. Also their regression analysis was based on patients treated with CDCA. In accordance with their results was our finding of a smaller percentage of CA in serum as compared with both dihydroxy-bile acids for reasons already mentioned.

It is clear that from our data no correlation with respect to pool size, synthesis, or fractional turnover of DCA can be drawn, since no kinetic experiments were done. It is, however, possible by using stable isotope techniques to gain insight into these parameters by serum bile acid analysis (20).

In conclusion, this study shows a significant and reproducible correlation between fasting serum and biliary DCA which can be a useful tool in human studies concerning DCA metabolism. Potential applicability could be the investigation of secondary bile acid metabolism in patients at high risk of developing colonic cancer and the manipulation of secondary bile acid formation by dietary means in these patients.

References

1. Breuer N, Goebell H. The role of bile acids in colonic carcinogenesis Klin Wochenstr 1985; 97-105.
2. MacDonald IA, Bokkenheuser VD, Winter J, McLernon AMj, Mosbach EH. Degradation of steriods in the human gut. J Lipid Res 1983; 24: 675-700.
3. Carey MC. The enterohepatic circulation. In: Arias I, Popper H, Schachter D and Shafritz DA eds. The liver: biology and pathobiology. Raven Press, New York 1982: 429-65.

4 Werf van der SDJ, Huybregts AMM, Lamers HLM, Berge Henegouwen van GP, Tongeren van JHM Age dependent differences in human bile acid metabolism and 7 α-dehydroxylation Eur J Clin Invest 1981, 11 425-31
5 Werf van der SDJ, Nagengast F M, Berge Henegouwen van GP, Huybregts AWM, Tongeren van JHM Colonic absorption of secondary bile acids in patients with adenomatous polyps and matched controls Lancet 1982, I 759 62
6 Nagengast FM, Werf van der SDJ, Lamers HLM, Hectors MPC, Buys WCAM, Tongeren van JHM Influence of age, intestinal transit time, and dietary composition on fecal bile acid profiles in healthy man Dig Dis Sci 1988 33, 673 8
7 Whiting MJ, Watts JMCK Prediction of the bile acid composition of bile from serum bile acid analysis during gallstone dissolution therapy Gastroenterology 1980, 78 220-5
8 Whiting MJ, Down RHL, Watts JMCK Chenodeoxycholic acid administration monitored by serum bile acid profiles a dose-response study Ann Clin Biochem 1983, 20 336-40
9 Bazzoli F, Fromm H, Roda A, Tunuguntla AK, Roda E, Barbara L, Amin P Value of serum determinations for prediction of increased ursodeoxycholic and chenodeoxycholic levels in bile Dig Dis Sci 1985, 7 650-54
10 Nagengast FM, Hectors MPC, Buys WCAM, Tongeren van JHM Inhibition of secondary bile acid formation in the large intestine by lactulose in healthy subjects of two different age groups Eur J Clin Invest 1988, 18 56-61
11 Tangerman A, Schaik van A, Hoek van der FW Analysis of conjugated and unconjugated bile acids in serum and jejunal fluid of normal subjects Clin Chim Acta 1986, 159 123-32
12 Setchell KDR, Matsui A Serum bile acid analysis Clin Chim Acta 1983, 127 1-17
13 Shaw R, Elliot WH Bile acids LV 2,2-Dimethoxypropane as esterifying agent preferred to diazomethane for chenodeoxycholic acid J Lipid Res 1978, 19 783 7
14 Huijbregts AWM, Schaik van A, Berge Henegouwen van GP, Werf van der SDJ Serum lipids, biliary lipid composition and bile acid metabolism in vegetarians as compared to normal controls Eur J Clin Invest 1980, 10 443 50
15 Dietschy JM Mechanisms for the intestinal absorption of bile acids J Lipid Res 1968, 9 297-309
16 Angelin B, Borkhem I Postprandial serum bile acids in healthy man Evidence for differences in absorptive pattern between individual bile acids Gut 1977, 18 606-9
17 Ahlberg J, Angelin B, Bjorkhem I, Einnarsson K Individual bile acids in portal venous and systemic blood serum of fasting man Gastroenterology 1977, 73 1377 82
18 Stellaard F, Paumgartner G A new model to assess deoxycholic acid metabolism in health using stable isotope dilution technique Eur J Clin Invest 1987, 27 63-7
19 Hofmann AF, Cravetto G, Molino G, Belforte G, Bona B Simulation of the metabolism and enterohepatic circulation of endogenous deoxycholic acid in humans using a physiologic pharmacokinetic model for bile acid metabolism Gastroenterology 1987, 93 693-709
20 Stellaard F, Paumgartner G, Berge Henegouwen van GP, Werf van der SDJ Determination of deoxycholic acid pool size and input rate using (24 ^{13}C) deoxycholic acid and serum sampling J Lipid Res 1986, 27 1222-25

Summary and conclusions

Summary

Chapter I presents an overview of the putative role of secondary bile acids in colonic carcinogenesis. Epidemiological observations indicate that a high dietary fat intake is associated positively and a high fibre intake negatively with colorectal cancer prevalence. These studies cannot clarify whether this association is causal and if so, by which mechanisms these dietary factors exert their action. An aspect of diet which is regarded important is the influence on secondary bile acid formation.

Secondary bile acids are formed by enzymatic deconjugation and 7 α-dehydroxylation of the primary bile acids by the anaerobic flora of the large bowel. The main secondary bile acids are deoxycholic acid (DCA) and lithocholic acid (LCA). DCA is partly absorbed in the colon and enters the enterohepatic circulation. LCA is almost insoluble and very little is absorbed. More than 90% of the bile acids excreted in the faeces are the unconjugated bile acids DCA and LCA.

Secondary bile acids can act as tumour promoters in chemically induced carcinogenesis in animal models and are co-mutagenic. Furthermore, colonic epithelial, proliferation can be enhanced by these bile acids. In epidemiological studies the prevalence of colonic cancer is associated with high faecal secondary bile acid concentrations. Case-control studies have given conflicting results in this respect, partly because of methodological problems.

Theoretically, inhibition of secondary bile acid formation should lead to a lower colonic cancer prevalence. In animal experiments manipulation of diet can reduce secondary bile acid levels in faeces and simultaneously the colon tumour yield. In man, secondary bile acid concentrations in bile and faeces can be lowered by adding bran to the diet. Lactulose, a non-absorbable disaccharide, which is fermented in the large bowel and reduces colonic pH, inhibits secondary bile acid formation. Diets rich in natural fibre can reduce faecal secondary bile acid concentrations but an inhibiting effect on the 7α-dehydroxylation of primary bile acids has not been shown yet.

At this moment the evidence for a promoting role of secondary bile acids in the carcinogenesis of large bowel tumours is largely circumstantial. Prospective dietary intervention studies in subjects at high risk for the development of this tumour may provide more insight in the capability of dietary factors to influence colonic carcinogenesis, and the role of secondary bile acids in this process.

Chapter II describes the methodology for the determination of faecal bile acids by gas-liquid chromatography. After refluxing freeze-dried faeces in an alkaline

milieu of methanol, it is possible to extract neutral steroids in petroleum ether. The acidic steroids (bile acids) left behind are simultaneously solvolysed and methylated by adding hydrochloric acid and dimethoxypropane. After extraction of the methylated bile acids with diethyl-ether and derivatisation into trifluoroacetates, separation and quantitation by gas-liquid chromatography is possible. The major primary and secondary bile acids can be separated and quantitated. Recoveries of added radiolabelled reference bile acids: chenodeoxycholic, deoxycholic and lithocholic acid were sufficient (85.8±2.6, 86.3±1.7 and 89.7±1.6%; ±SD, respectively) and reproducible. The intra-assay precision for the determination of the most prominent faecal bile acids LCA and DCA was 2.8 and 4%, respectively. The inter-assay precision was 9.0 and 7.2% for LCA and DCA.

Chapter III describes the influence of age and change in nutrient consumption on faecal bile acid profiles in healthy subjects is described. The concentration of the major faecal secondary bile acids increased with advancing age and was higher in elderly subjects compared to young adults. Analysis of dietary constituents showed that elderly subjects consumed less fibre and had a lower dry faecal material excretion than both young adults and middle-aged persons. This could well explain the increase in the faecal bile acid concentration with advancing age. Moreover, dietary fibre intake was negatively correlated to the total bile acid concentration. The results showed that an age-dependent increase in faecal secondary bile acid concentration can occur and that this phenomenon is related to changes in dietary habits. Thus, matching for age is important when case-control studies concerning the role of faecal bile acids in colorectal carcinogenesis are conducted.

Chapter IV presents the results of a study to determine the effects of lactulose, a nonabsorbable disaccharide, on deoxycholic acid formation in healthy subjects of two different age groups. Lactulose administration for 12 weeks reduced the biliary DCA content by 25% and increased the primary bile acid output in the stool to more than 20%. The whole gut transit time was not altered. The faecal pH dropped after 6 and further after 12 weeks lactulose. The results suggest that lactulose inhibits 7α-dehydroxylation of primary bile acids, probably because of acidification of colonic contents.

Since lactulose is not a natural compound that can be incorporated into the diet, we have examined the effects of a natural high fiber diet on faecal and biliary bile acids, whole gut transit time and faecal pH in a controlled study. The results of this study are shown in *Chapter V*.
Healthy subjects (12) with an habitual low fibre intake increased their fibre con-

sumption from various sources. Care was taken to keep the intake of all other nutrients (especially fat) unaltered. Since not much is known about the physiological variations of bile acid metabolism when a stable diet is consumed, a control group with a habitual high fibre intake was included.
The concentration of the major faecal secondary bile acids decreased significantly in the experimental group as compared with changes in the control group. No increase of the fraction of primary bile acids occurred. The total faecal bile acid excretion did not change in the experimental group as compared with the control group. The whole gut transit time did not decrease significantly, in contrast to the faecal pH after 10 weeks in the experimental group, in comparison with changes in the control group. The biliary DCA content in the experimental group decreased after 6 weeks in comparison with the change in the control group and the proportion biliary CA increased significantly in this period. However, after 10 weeks of high fibre diet, biliary DCA increased slightly and the CA content in bile returned to the initial value.
The results show that the faecal secondary bile acid concentration can be reduced on a natural high fibre diet in man. The effect is mediated through an increase in stool wet and dry weight. The 7α-dehydroxylation of primary bile acids, however, is probably not or only transiently inhibited.

The DCA content of bile is a reflection of the formation and absorption of this bile acid in the large bowel. It can therefore be used as a marker for the exposure of the colonic mucosa to secondary bile acids. To investigate biliary bile acids, duodenal intubation is a prerequisite and this invasive procedure limits its application in human studies on a larger scale. Serum bile acid analysis would overcome this drawback if a significant and reproducible correlation with biliary bile acid composition would be present. *Chapter VI* presents the results of a study in which this relationship was examined for the three major bile acids cholic acid (CA), chenodeoxycholic acid (CDCA) and DCA. The study was performed in the same healthy volunteers who took part in the lactulose study from chapter IV. On two consecutive weeks before and after 6 and 12 weeks of lactulose administration, fasting bile and serum samples were obtained simultaneously. Serum and biliary bile acids were analysed by capillary gas-liquid chromatography.
The study showed that an acceptable separation and quantitation of serum bile acids was possible by using this method. A significant and reproducible correlation was found between the molar percentages of DCA in bile and serum both before and during lactulose (correlation coëfficients >0.90; $p<0.001$). The equation of the correlation was: Y (biliary DCA) = 2.7+0.56 X (serum DCA). Within 8% variation the molar percentage of DCA in bile could be predicted from the serum value using this equation. The reduction of the biliary DCA content during lactulose was equalled by the decline in the molar percentage of DCA

in serum after 6 and 12 weeks. The correlation for the other bile acids varied from 0.53 to 0.67 for CA and from 0.63 to 0.92 for CDCA.

The results of this study show that the molar percentages of biliary and serum DCA are closely interrelated and that changes in biliary DCA can be predicted from serum DCA analysis.

Potential applicability could be the investigation of DCA metabolism in patients at high risk of developing colonic cancer, and modulation of DCA metabolism by dietary means.

Conclusions

From data in the literature and studies presented in this thesis it is clear that secondary bile acid metabolism can be influenced by dietary changes. The formation, absorption and faecal excretion of the secondary bile acid deoxycholic acid (DCA) increases with advancing age and is related to the dietary intake of fibre. Inhibition of 7α-dehydroxylation of cholic acid (CA), inducing a decreased conversion of CA into DCA, is possible by acidification of colonic contents. However, consumption of a natural high fibre diet does not lead to such a substantial decline of the colonic pH to inhibit 7α-dehydroxylase. The complex nature of fibre most likely explains this finding. Fibre consists of many different components, which can exert opposite effects on the colonic flora and also on bile acid metabolism. Since a high fibre intake increases the output of both stool wet and dry material, the concentrations of the major secondary bile acids in the faeces and probably also in the colonic lumen will be reduced.

Whether these effects will lead to a reduction in colonic cancer prevalence has yet to be proven. Experiments in which the dynamics of the enterohepatic circulation of DCA are investigated (by isotope dilution by which pool size, fractional turnover, synthesis and/or input can be calculated) on diets with different fibre contents could elucidate the mechanism by which fibre exerts its action.

A prospective dietary intervention trial in patients with an increased risk of developing colonic cancer could answer the question whether diet really plays an important role in the causation of this disease.

Samenvatting en conclusies

Samenvatting

In *hoofdstuk I* wordt een overzicht gegeven van de mogelijke rol die secundaire galzuren spelen in het ontstaan van coloncarcinoom. Uit epidemiologisch onderzoek is gebleken, dat consumptie van vet positief en van vezel negatief is gecorreleerd aan de prevalentie van coloncarcinoom. Dit onderzoek geeft geen opheldering over een oorzakelijk verband en, indien een dergelijke relatie zou bestaan, op welke wijze deze voedingsfactoren hun effect bewerkstelligen. Een aspect van voeding dat belangrijk wordt geacht is de invloed op de vorming van secundaire galzuren.
Secundaire galzuren worden gevormd na enzymatische deconjugatie en 7α-dehydroxylatie van de primaire galzuren door de anaerobe flora van de dikke darm. De belangrijkste secundaire galzuren bij de mens zijn deoxycholzuur (DCA) en lithocholzuur (LCA). Deoxycholzuur wordt gedeeltelijk geabsorbeerd in het colon en komt aldus in de enterohepatische kringloop terecht. Lithocholzuur is vrijwel onoplosbaar en zeer weinig wordt geresorbeered. De galzuren die worden uitgescheiden in de ontlasting, bestaan voor meer dan 90% uit ongeconjugeerd deoxy- en lithocholzuur.
Secundaire galzuren kunnen een rol spelen als promoverende factoren in door chemische stoffen opgewekte colontumoren bij proefdieren en zijn bovendien co-mutageen. De proliferatie van colonepitheel kan door deze galzuren worden versterkt. De prevalentie van coloncarcinoom heeft een relatie met hoge concentraties van secundaire galzuren in de ontlasting. Studies waarin patiënten met coloncarcinoom werden vergeleken met gezonde controle personen, hebben wat dit betreft tegengestelde resultaten opgeleverd, hetgeen gedeeltelijk te verklaren is door methodologische problemen in de faecale galzuur bepaling.
Theoretisch zou een remming van de vorming van secundaire galzuren moeten leiden tot een daling in de prevalentie van colontumoren. In onderzoek met proefdieren kan de faecale concentratie van secundaire galzuren worden verlaagd en het aantal colontumoren afnemen door veranderingen in de voedingsamenstelling. Bij de mens kan de hoeveelheid secundaire galzuren in de gal en de ontlasting afnemen door zemelen aan de voeding toe te voegen. Lactulose, een disaccharide dat niet in de dunne darm wordt gesplitst en geabsorbeerd, wordt in het colon afgebroken door de bacteriele flora in korte keten vetzuren. Hierdoor ontstaat een daling van de intraluminale pH in de darm, hetgeen de 7α-dehydroxylatie van primaire galzuren remt en zo de vorming van secundaire galzuren. Voedingen die rijk zijn aan natuurlijke vezels kunnen de concentatie van secundaire galzuren in de faeces doen dalen, echter of de vorming van deze galzuren afneemt, is nog omstreden.

Op dit ogenblik bestaan er alleen indirecte aanwijzingen, dat secundaire galzuren een promoverende rol spelen in het ontstaan van coloncarcinoom. Prospective interventie studies met voedingen van verschillende samenstelling bij personen met een hoog risico op het ontstaan van deze tumoren, zou meer inzicht kunnen verschaffen in het effect van voedingsfactoren op de coloncarcinogenese en de rol van galzuren hierin.

In *hoofdstuk II* wordt de methode beschreven waarmee de faecale galzuren met behulp van gaschromatografie kunnen worden bepaald.
Nadat gevriesdroogde faeces in een alkalisch milieu van methanol een reflux procedure heeft ondergaan om de galzuren los te maken van de aanwezige droge stof, kunnen neutrale steroïden worden geëxtraheerd in petroleumether. De zure steroïden (galzuren) die achter zijn gebleven, worden dan gelijktijdig gedesulfateerd en gemethyleerd door zoutzuur en dimethoxypropaan aan de oplossing toe te voegen. Nadat de gemethyleerde galzuren met diethylether zijn geëxtraheerd, worden er trifluoroacetaat verbindingen van gemaakt, die dan met behulp van de gaschromatograaf worden gescheiden en gekwantificeerd. De belangrijkste primaire en secundaire galzuren kunnen aldus worden gescheiden en gekwantificeerd. De opbrengst van de toegevoegde radio-actieve referentie galzuren chenodeoxycholzuur, deoxycholzuur en lithocholzuur was voldoende (respectievelijk 85.8±2.6, 86.3±1.7 en 89.7±1.6%) en reproduceerbaar. De variatie binnen een bepaling voor de belangrijkste faecale galzuren lithocholzuur en deoxycholzuur was respectievelijk 2.8 en 4.0%. De variatie tussen verschillende bepalingen was 9.0 en 7.2% voor litho- en deoxycholzuur.

In *hoofdstuk III* wordt de invloed van leeftijd en daarmee samenhangende verandering in voedingsgewoontes op de faecale galzuur profielen beschreven. De concentratie van de belangrijkste faecale secundaire galzuren nam toe met de leeftijd en was hoger bij oudere personen dan bij jonge volwassenen. Analyse van voedingsgegevens liet zien dat oudere personen minder vezel consumeerden. Zij scheidden minder droog faeces materiaal uit dan zowel jonge volwassenen als personen van middelbare leeftijd. Dit zou goed de verklaring kunnen zijn voor de toename in de secundaire galzuurconcentratie bij oudere personen. Bovendien was de hoeveelheid vezel die werd gebruikt negatief gecorreleerd met de totale faecale galzuurconcentratie.
De resultaten van deze studie toonden een leeftijdsafhankelijke toename in de concentratie van secundaire galzuren in de ontlasting aan en een relatie met veranderingen in voedingsgewoontes. Het is daarom belangrijk dat in studies waarin faecale galzuren worden gemeten bij patiënten met colontumoren en gezonde controle personen, de groepen qua leeftijd vergelijkbaar zijn.

In *hoofdstuk IV* worden de resultaten beschreven van een onderzoek om de effecten van lactulose (een niet in de dunne darm gesplitst of geabsorbeerd disaccharide) op de vorming van deoxycholzuur te meten bij gezonde personen van twee verschillende leeftijds categorieën. Toediening van lactulose gedurende 12 weken deed het gehalte aan deoxycholzuur in de gal met 25% dalen en de excretie van primaire galzuren in de ontlasting tot ruim 20% toenemen. De totale darmpassagetijd veranderde niet. De pH van de faeces daalde na 6 en verder na 12 weken lactulose toediening. Deze resultaten geven aan dat de 7α-dehydroxylatie van primaire galzuren wordt geremd door lactulose, waarschijnlijk als gevolg van aanzuring van de inhoud van de dikke darm.

Aangezien lactulose geen natuurlijk voorkomende voedingsstof is die kan worden toegevoegd aan een normale voeding, zijn de effecten van een natuurlijke, vezelrijke voeding op de galzuursamenstelling van faeces en gal, op de totale darmpassagetijd en op de faecale pH onderzocht in een gecontroleerde studie. De resultaten worden beschreven in *hoofdstuk V*.
Gezonde vrijwilligers die een relatief vezelarme voeding gebruikten, verhoogden hun vezelconsumptie (afkomstig van zowel granen, fruit als groente) gedurende 10 weken. Er werd nauwlettend op toegezien, dat er in de consumptie van andere voedingsstoffen (speciaal vet) niets veranderde. Een groep personen die een relatief vezelrijke voeding gebruikte en deze gedurende de gehele studie periode bleef gebruiken, fungeerde als controle groep.
De concentratie van de belangrijkste faecale secundaire galzuren daalde significant in de experimentele groep, vergeleken met veranderingen in de controle groep. De hoeveelheid primaire galzuren nam niet toe. De totale galzuuruitscheiding in de experimentele groep veranderde niet ten opzichte van veranderingen in de controle groep.
De totale darmpassagetijd veranderde niet, doch de faecale pH daalde na 10 weken in de experimentele groep, vergeleken met veranderingen in de controlegroep.
Het aandeel van deoxycholzuur in de galsamenstelling daalde na 6 weken in de experimentele groep, in vergelijking met de controlegroep en het aandeel van cholzuur steeg in deze periode. Echter, na 10 weken vezelrijke voeding, steeg het deoxycholzuur-gehalte licht en daalde het cholzuur-gehalte tot de uitgangswaarde.
De resultaten van deze studie laten zien, dat de concentratie van de faecale secundaire galzuren daalt tijdens een natuurlijke vezelrijke voeding. Dit effect berust op een toename in het natte en droge gewicht van de ontlasting. De 7α-dehydroxylatie van primaire galzuren wordt echter waarschijnlijk niet of slechts tijdelijk geremd.

Het gehalte deoxycholzuur in gal weerspiegelt de vorming en absorptie van dit galzuur in de dikke darm. Het kan daarom gebruikt worden als de mate waarin de colonmucosa wordt blootgesteld aan secundaire galzuren. Om de galzuursamenstelling van de gal te kunnen onderzoeken, is intubatie van het doudenum noodzakelijk. Het feit dat dit een invasieve procedure is, beperkt de toepassing ervan op grotere schaal in humane studies. Serum galzuur analyse zou dit bezwaar kunnen ondervangen, als er een significante en reproduceerbare correlatie met de galzuursamenstelling in de gal zou bestaan.

In *hoofdstuk VI* worden de resultaten beschreven van een onderzoek, waarin de relatie tussen de belangrijkste galzuren: cholzuur, chenodeoxycholzuur en deoxycholzuur in serum en gal werd nagegaan. Het onderzoek werd uitgevoerd bij dezelfde gezonde vrijwilligers die deelnamen aan de lactulose studie uit hoofdstuk IV. Op twee achtereenvolgende weken voor en na 6 en 12 weken lactulose toediening werden gelijktijdig nuchtere gal- en serum-monsters afgenomen. Serum-galzuren werden bepaald middels capillaire gaschromatografie. Het bleek dat de galzuren in serum met deze methode betrouwbaar gescheiden en gekwantificeerd konden worden.

Er werd een significante en reproduceerbare correlatie gevonden tussen de molaire percentages deoxycholzuur in serum en gal, zowel voor als tijdens toediening van lactulose (correlatie coëfficiënten >90%; $p<0.001$). De vergelijking van de correlatie was: Y (DCA in gal) = 2.7 + 0.56 X (DCA in serum). Met behulp van deze vergelijking kon het molair percentage van deoxycholzuur in gal worden voorspeld uit de serum-waarde met een varatie van 8%. De afname van het gehalte deoxycholzuur in gal gedurende lactulose weerspiegelde zich in een evenredige afname in serum na 6 en 12 weken. De correlatie tussen gal en serum voor de andere galzuren varieerde van 0.53 tot 0.67 voor cholzuur en van 0.63 tot 0.92 voor chenodeoxycholzuur.

De resultaten van deze studie laten zien dat de molaire percentages van het gehalte deoxycholzuur in gal en serum nauw met elkaar samenhangen en dat veranderingen in het percentage deoxycholzuur in gal voorspelt kunnen worden uit de veranderingen in het serum deoxycholzuur gehalte.

Potentiële toepassing zou kunnen bestaan uit het bestuderen van het deoxycholzuur metabolisme bij patiënten met een verhoogd risico op het ontstaan van coloncarcinoom en het beïnvloeden van het deoxycholzuur metabolisme via veranderingen in het voedingspatroon.

Conclusies

Uit de literatuur en studies in dit proefschrift is het duidelijk, dat het metabolisme van secundaire galzuren kan worden beïnvloed via veranderingen in de voeding. De vorming, absorptie en faecale excretie van het secundaire galzuur deoxycholzuur neemt toe met het stijgen van de leeftijd en is gerelateerd aan de consumptie van vezels. Remming van de 7α-dehydroxylatie van cholzuur, met als gevolg een afgenomen omzetting van cholzuur in deoxycholzuur, is mogelijk door aanzuring van de colon inhoud. Echter het gebruik van een voeding rijk aan natuurlijke vezels leidt niet altijd tot een zodanige daling van de pH in het colon, dat het enzym 7α-dehydroxylase wordt geremd. De complexiteit van vezel is de meest voor de hand liggende verklaring van deze bevinding. Vezelstoffen bestaan uit vele verschillende verbindingen die uiteenlopende en tegengestelde effecten kunnen hebben op de bacteriële flora in het colon en ook op het metabolisme van galzuren. Aangezien een grote vezelconsumptie de uitscheiding van zowel natte als droge stof in de ontlasting doet toenemen, zullen de concentraties van de secundaire galzuren in de faeces en waarschijnlijk ook in het colon afnemen.
Het moet nog worden aangetoond, dat deze effecten zullen leiden tot een daling in de prevalentie van colon carcinoom. Experimenten waarin de dynamiek van de enterohepatische circulatie van deoxycholzuur wordt bestudeerd (middels isotopen-dilutie, waardoor de grootte van de voorraad, de halfwaardetijd, de synthese en/of de absorptie kan worden berekend), met behulp van voedingen met verschillende vezelgehalten zou meer inzicht kunnen verschaffen in het werkingsmechanisme van deze verbindingen.
Een prospectief opgezet voedingsinterventie-onderzoek bij patiënten met een verhoogd risico op het ontstaan van coloncarcinoom kan de vraag beantwoorden of voeding werkelijk een belangrijke rol speelt in het ontstaan van deze ziekte.

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Curriculum vitae

De auteur van dit proefschrift werd geboren op 6 februari 1950 te Groningen. In 1967 werd het eindexamen HBS-B behaald aan het Heymans Lyceum te Groningen. Aansluitend studeerde hij geneeskunde aan de Rijksuniversiteit te Groningen, behaalde het doctoraalexamen in 1972 en het artsexamen in 1974. Vervolgens was hij tot september 1975 werkzaam als arts-assistent op de afdeling Inwendige Geneeskunde van het Streekziekenhuis Midden Twente (destijds: Koningin Juliana Ziekenhuis; internisten H.K. van Schothorst en A.J. te Rijdt). Van september 1975 tot november 1978 volgde hij de opleiding tot internist in het St. Geertuiden Ziekenhuis te Deventer (opleider: J.H. Scholten, internist). De opleiding werd vanaf november 1978 voortgezet aan de Universiteitskliniek voor Inwendige Ziekten van het Sint Radboudziekenhuis te Nijmegen (hoofd destijds: Prof. Dr. C.L.H. Majoor† en thans Prof. Dr. A. van 't Laar). Op 1 september 1980 werd hij ingeschreven als internist in het specialistenregister.
Vanaf 1981 is hij verbonden aan de afdeling Maag-, Darm- en Leverziekten (hoofd: Dr. J.H.M. van Tongeren). In oktober 1985 werd de opleiding tot gastroenteroloog voltooid. Hij is getrouwd met Riekje Kooi en zij hebben 3 kinderen: Annemiek, Wouter en Marleen.

Stellingen

1. De concentratie van secundaire galzuren in de ontlasting stijgt met de leeftijd en hangt samen met een verandering in voedingsgewoontes.
   – *dit proefschrift* –

2. Door voldoende aanzuring van de inhoud van de dikke darm wordt de 7α-dehydroxylatie van primaire galzuren geremd en aldus de vorming van secundaire galzuren.
   – *dit proefschrift* –

3. Consumptie van een voeding rijk aan vezels uit diverse bronnen leidt wel tot een daling van de concentratie van secundaire galzuren in de ontlasting, doch waarschijnlijk niet tot een remming van de vorming van deze galzuren.
   – *dit proefschrift* –

4. Als preventief oncologische behandeling van patiënten met polyposis coli moet het uitvoeren van een totale colectomie met aanleggen van een ileo-anale anastomose als eerste keus behandeling worden beschouwd.

5. Bij het opsporen en in kaart brengen van families met een erfelijke vorm van coloncarcinoom is de hulp van de huisarts onmisbaar.

6. Bij patiënten met grote choledochusstenen die niet endoscopisch verwijderd kunnen worden, kan aanvullende extra-corporele lithotrypsie deze stenen zodanig fragmenteren, dat spontane uitdrijving alsnog plaatsvindt.
   T Sauerbruch et al. N Engl J Med 1986; 314: 818-22.
   SH Yap, FM Nagengast, eigen waarnemingen.

7. Het spastische-bekkenbodemsyndroom is een vaak niet herkende oorzaak van obstipatie en kan middels defaecografisch onderzoek betrekkelijk eenvoudig worden opgespoord.
   JHC Kuijpers, G Bleijenberg. Ned Tijdschr Geneesk 1985; 19:1624-8.

8. Als een patiënt met bloedende oesophagusvarices die wordt behandeld met vasopressine in coma raakt, moet niet alleen een coma hepaticum worden overwogen, doch ook aan een waterintoxicatie worden gedacht.
   SJ Graafsma, FM Nagengast, eigen waarnemingen.

9. Bij een patiënt met een hemodynamisch belangrijke aortastenose die recidiverend intestinale bloedingen heeft als gevolg van angiodysplasieën in de darm, kunnen deze bloedingen tot staan worden gebracht door het vervangen van de aortaklep door een prothese.
   MS Chappell, O Lebwohl. Ann Intern Med 1986; 105: 54-7.
   Eigen waarnemingen.

10. Ultrasonografie kan een waardevol hulpmiddel zijn bij de diagnostiek van acute appendicitis.
    JBCN Puylaert et al. N Eng J Med 1987; 317: 666-9.

11. In het voorkómen van bronchospasmen bij blootstelling aan een allergisch agens is de ontwikkeling van cromoglicaat een zeer belangrijke stap vooruit geweest voor patiënten met CARA.

12. Hoewel veelal toegeschreven aan Frederic Chopin (1810-1849), is de Ierse componist John Fields (1782-1837) de „uitvinder" van de nocturne.

13. Gepromoveerd zijn betekent ook: nooit meer 's nachts beklemd titelloos wakker worden en denken..... dr staat niets voor m'n naam!
    Vrij naar Wim Kan.

14. Wadlopen is meer dan wat lopen.

F.M. Nagengast

Nijmegen, 30 september 1988